

DRUG COMBINATION COMPRISING ANTI-CD37 ANTIBODY MAYTANSINE
CONJUGATE AND BCL2 INHIBITOR OR RISK
INHIBITOR

DESCRIPTION

5 The present invention relates to drug combinations for the treatment of disease. In particular, the present invention relates to combinations of ligand-drug-conjugates with BCL2 inhibitors or PI3K pathway inhibitors and uses thereof in the treatment of cancer, and especially diffuse large B-cell lymphoma (DLBCL).

10 BACKGROUND OF THE INVENTION

Various therapeutic approaches for the treatment of cancer are known, including antibody drug conjugates such as the trastuzumab-based commercial product Kadcyla®. There is nevertheless an urgent need for further effective cancer therapies.

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SUMMARY OF THE PRESENT INVENTION

The present invention provides novel drug combinations. The combinations are suitable for use in cancer therapy and especially for the treatment of DLBCL.

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The invention thus provides the following:

1. A combination comprising a conjugate compound of the following formula (I) or pharmaceutically acceptable salt, solvate or polymorph thereof, and a co-agent
25 selected from a BCL2 inhibitor or pharmaceutically acceptable salt, solvate or polymorph thereof and a PI3K pathway inhibitor or pharmaceutically acceptable salt, solvate or polymorph thereof, wherein the formula (I) is



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wherein V represents an anti-CD37 antibody, a fragment or derivative thereof or Fc-fusion protein containing an anti-CD37 antibody fragment and preferably naratuximab, a fragment or derivative thereof or Fc-fusion protein containing a naratuximab fragment,

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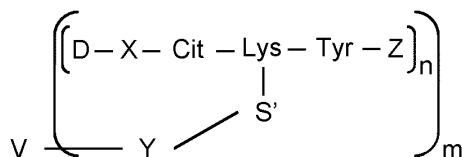
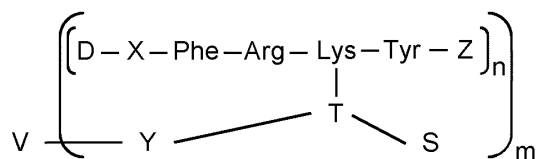
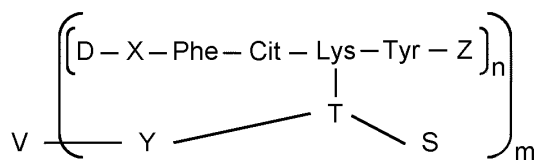
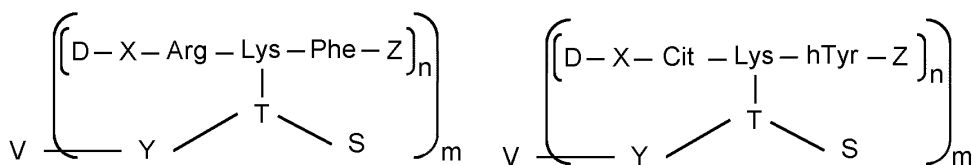
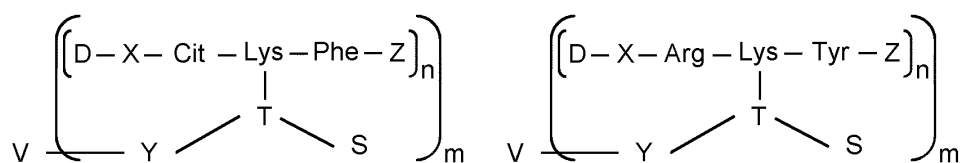
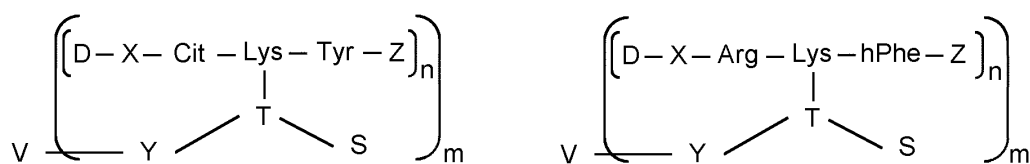
LL represents a linker covalently bonded to V and D,

D represents a moiety derived from a drug selected from maytansine, mertansine (DM1) and raptansine (DM4), preferably DM1 or DM4 and more preferably DM1; and

m is an integer of 1 to 12, preferably 2 to 10 and more preferably 4 to 8.

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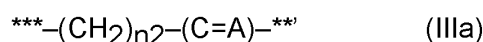
2. The combination of item 1, wherein the conjugate compound of formula (I) is a compound represented by one of the following formulae:



wherein

D has the same meaning as specified in claim 1, and preferably D is a moiety derived from DM1 or DM4;

X is a group of formula (IIIa),

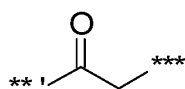


wherein n_2 is 1 or 2,

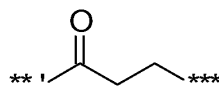
A is selected from O and S, preferably O;

*** represents covalent attachment to D; and

**' represents covalent attachment to the adjacent amino acid Arg, Cit or Phe; preferably a group represented by formula (IVc) or (IVd)



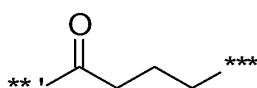
(IVc)



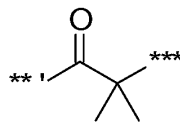
(IVd)

or

X is a group represented by formula (IVe) or (IVf)



(IVe)



(IVf)

*** represents covalent attachment to D;

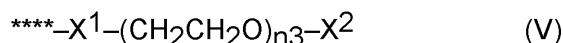
**' represents covalent attachment to the adjacent amino acid Arg, Cit or Phe;

Y is a group derived from a compound selected from maleimides, triazoles, hydrazones, carbonyl-containing compounds and derivatives thereof, preferably from maleimides and derivatives thereof such as opened hydrolyzed maleimide derivatives, and more preferably derived from an opened hydrolyzed maleimide;

T is derived from an amino acid selected from N-ε-propargyloxycarbonyl-L-Lysine (Lys(Poc)), Asp, Glu, Orn, Lys, Dab

and Dap, preferably derived from Lys(Poc), Glu, Orn or Lys, more preferably derived from Lys;

S is a moiety of formula (V)



wherein

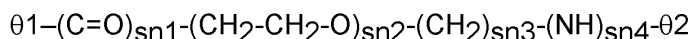
$n3$ is an integer of 6 to 200, preferably 10 to 150, more preferably 12 to 80;

**** indicates covalent attachment to T, preferably the side chain of T;

X^1 is selected from a single covalent bond, $-(C=O)-$, and $-N(R)-$ in which R represents a hydrogen atom, an alkyl group or a cycloalkyl group;

X^2 represents an alkyl group having 1 to 6 carbon atoms, a carbonyl-containing group such as an acetyl group or a group of formula $-(CH_2)_{n4}-CO_2H$, a thiocarbonyl-containing group, a group of formula $-(CH_2)_{n4}OR$, a group of formula $-(CH_2)_{n4}-SO_3H$, or an amino-containing group such as a group of formula $-(CH_2)_{n4}-(C=A)-N(R)_2$ or $-(CH_2)_{n4}-N(R)_2$, in which A is O or S, each R is independently selected from a hydrogen atom, an alkyl group and a cycloalkyl group, and $n4$ is an integer of 1 to 6

S' is a moiety represented by the following formula

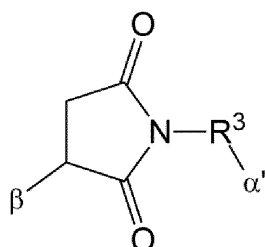


wherein $sn1$ and $sn4$ are independently selected from 0 and 1, $sn2$ is selected from 2 to 8, preferably 3 to 7 and more preferably 4 to 6, and $sn3$ is selected from 1 and 2, wherein most preferably $sn1$ and $sn4$ are each 1, $sn2$ is 5, and $sn3$ is 2, and wherein $\theta1$ is the position of a covalent bond to the sidechain of Lys and $\theta2$ is the position of a covalent bond to Y;

Z is -OH; and

n is 1.

- 5 3. The combination of item 2, wherein group S in the conjugate compound of formula (I) is represented by a moiety of formula (V), wherein X^1 is $-(C=O)-$, X^2 is a methyl group and n_3 is selected from the range of 14 to 20, preferably 16 to 18.
4. The combination of any one of items 2 and 3, wherein group Y in the conjugate compound of formula (I) is represented by a divalent group represented by any of the following formula (XIIIa):



Formula (XIIIa)

wherein,

- 15 R^3 represents $-(CH_2)_{n_7}-(C=A)_{n_9}-\alpha'$ or $-(CH_2)_{n_7}-(C=A)_{n_9}-NH-(CH_2CH_2O)_{n_8}-(C=A)_{n_9}-\alpha'$, preferably $-(CH_2)_{n_7}-(C=A)_{n_9}-\alpha'$, wherein,

n_7 is 1 or 2, preferably 1,

n_8 is 1 to 6, preferably 1,

n_9 is 0 or 1, preferably 1, and

- 20 A is O or S, preferably O;

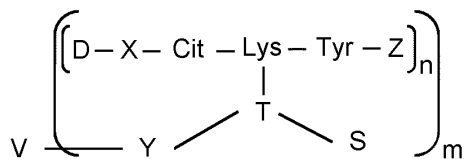
wherein the methylene carbon atom of R^3 is covalently attached to the nitrogen atom of formulae (XIIIa) and the carbonyl or thiocarbonyl-carbon is covalently attached to T;

β indicates covalent attachment to V; and

- 25 α' indicates covalent attachment to T, preferably by amide bond formation with the amino group that is attached to C α of the amino acid.

5. The combination of any one of items 2, 3 and 4, wherein the conjugate compound of formula (I) is a compound of the following structure:

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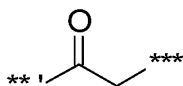


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wherein D, X, Z, T, Y, S, V, n and m are as defined in any of the preceding claims,
wherein preferably

10 D is a moiety derived from DM1;

X is a group represented by formula (IVc)



(IVc)

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*** represents covalent attachment to D;

** represents covalent attachment to the adjacent amino acid Cit;

20 Y is a group of formula (XIIIa) as defined in claim 4, wherein R³ represents $-(\text{CH}_2)_{n7}-(\text{C}=\text{A})_{n9}-\alpha'$, wherein,

n₇ is 1,

n₉ is 1, and

A is O;

25 wherein the methylene carbon atom of R³ is covalently attached to the nitrogen atom of formula (XIIIa) and the carbonyl-carbon is covalently attached to T, forming an amide bond with the Lys;

β indicates covalent attachment to V; and

α' indicates covalent attachment to T, preferably by amide bond formation with the amino group that is attached to Cα of the amino acid;

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T is derived from an amino acid selected from Lys(Poc), Glu, Orn or Lys, preferably derived from Lys;

S is as specified in item 3;

Z is -OH;

V is derived from naratuximab; and

n is 1.

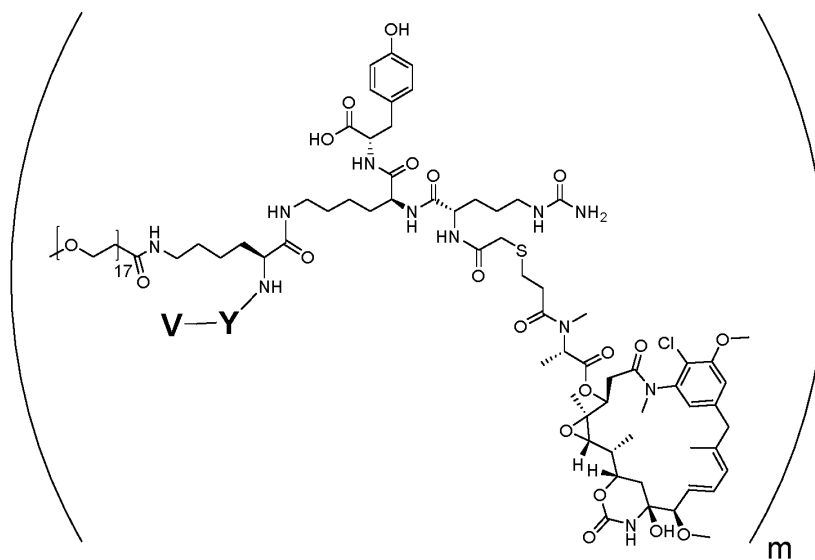
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6. The combination of any one of items 1 to 5, wherein the conjugate compound of formula (I) is represented by one of the following formulae:

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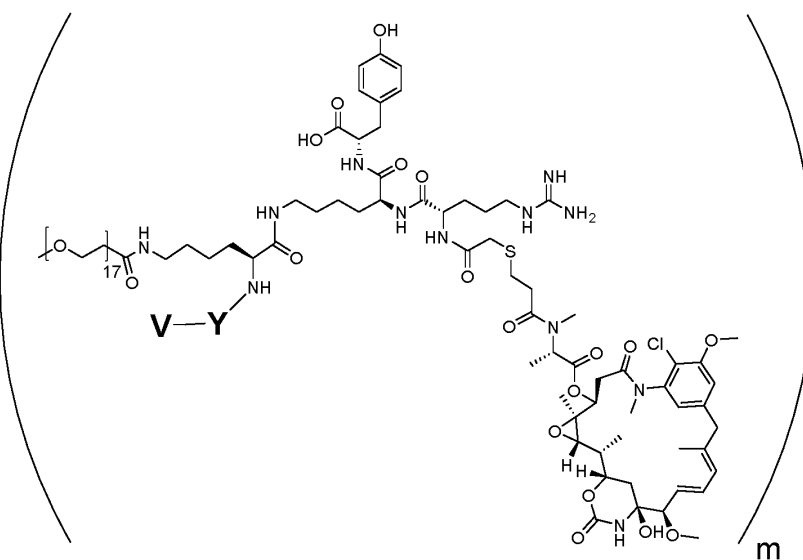
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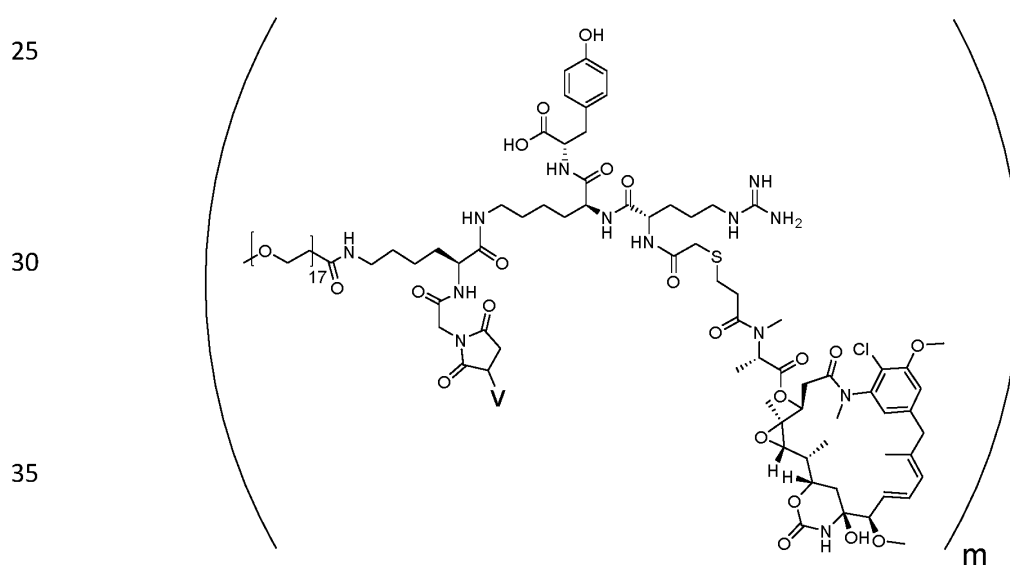
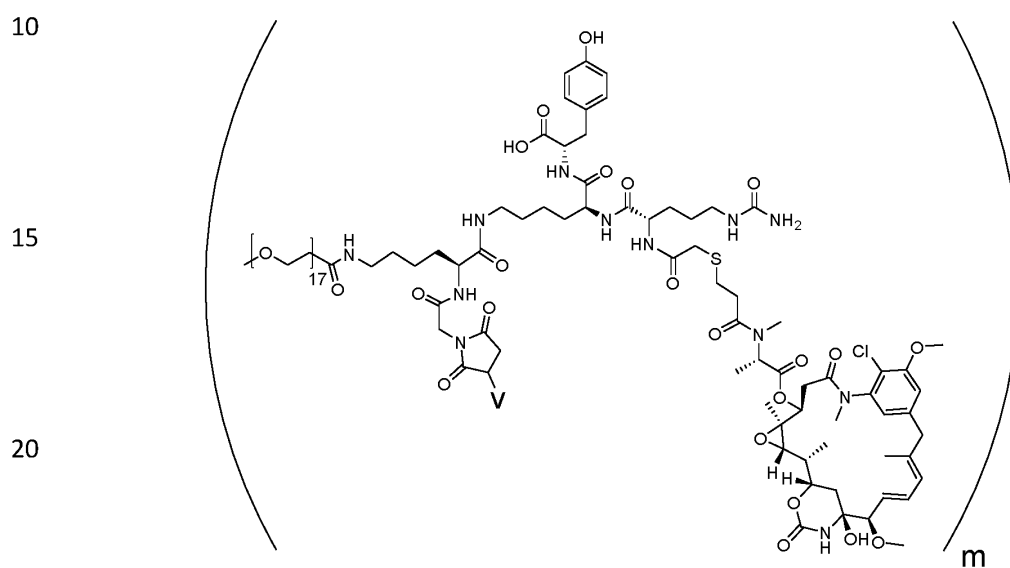
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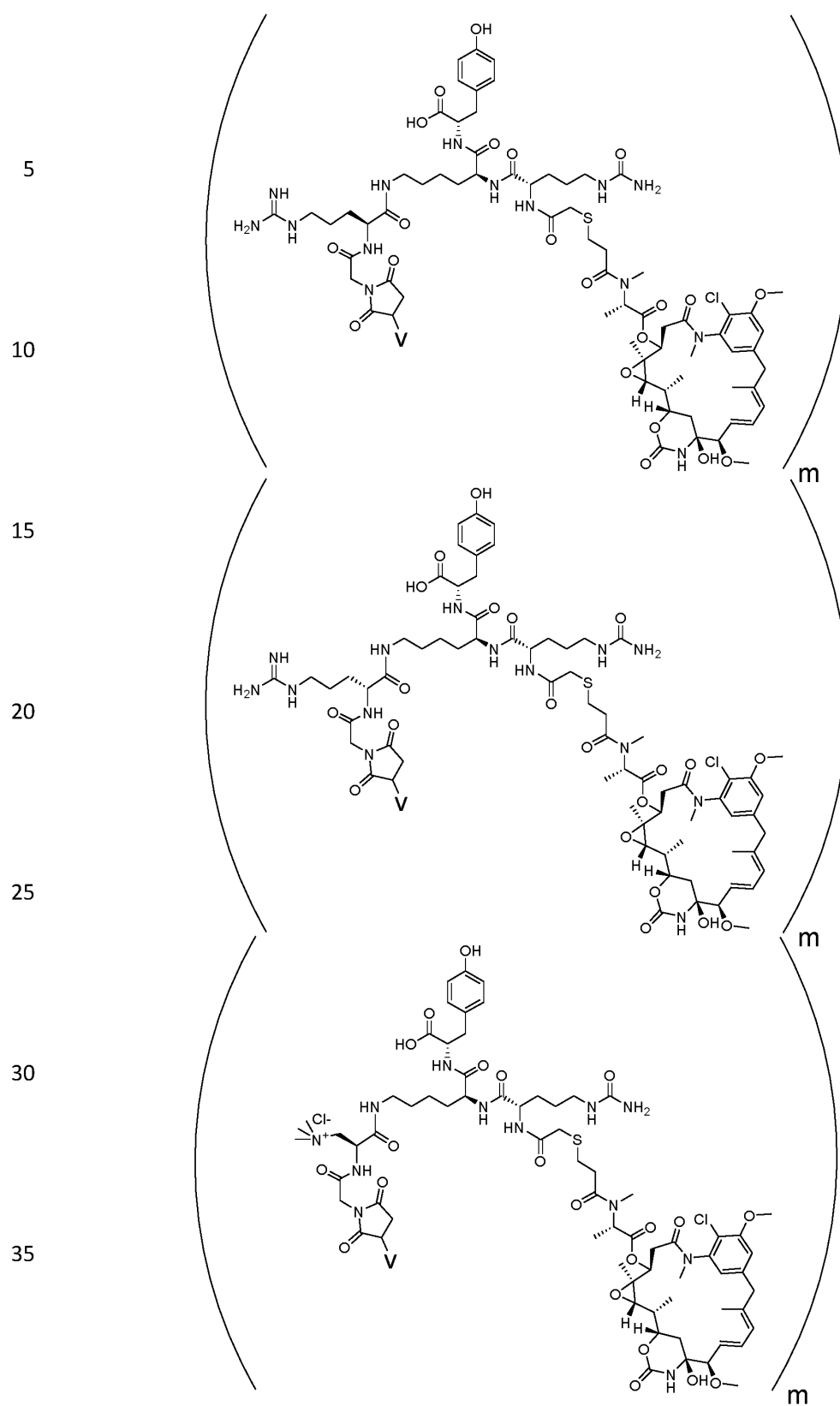


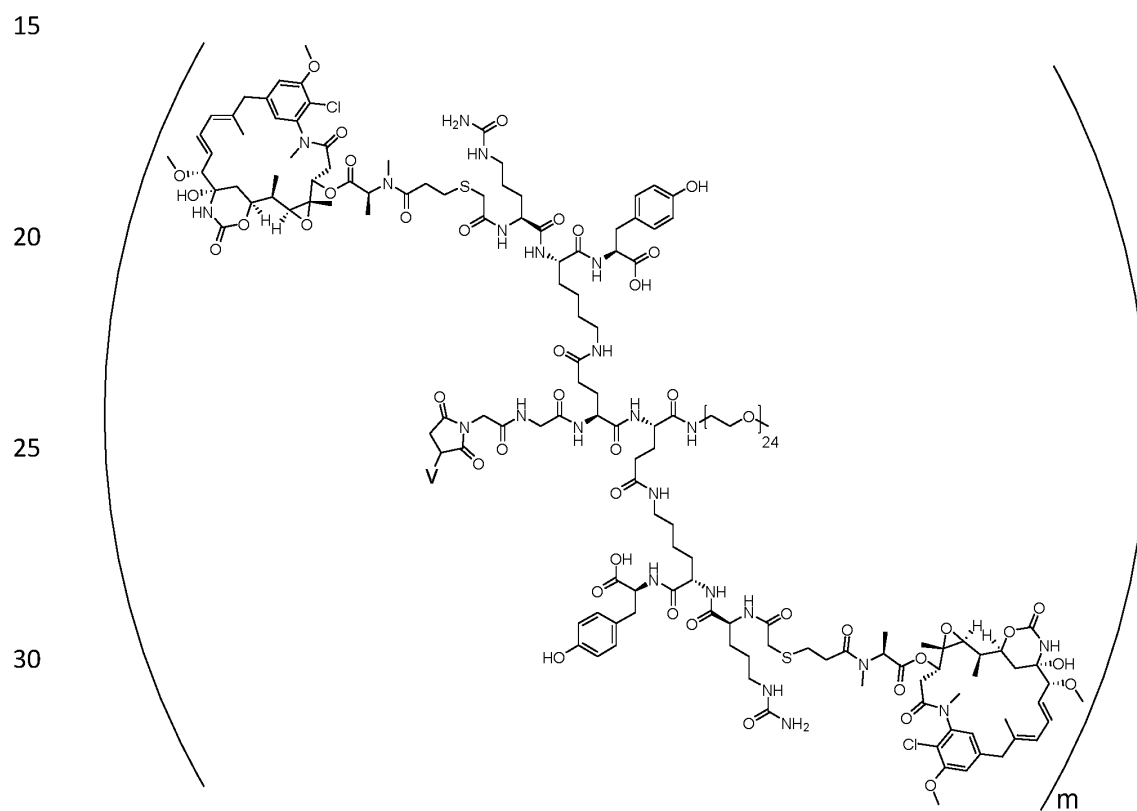
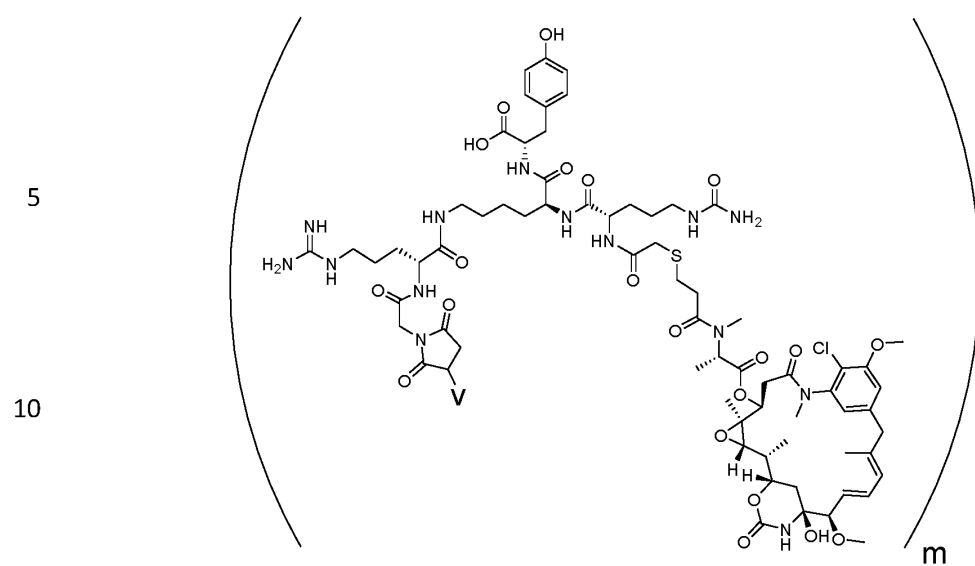
wherein Y, V and m are as defined in any of the preceding claims, preferably wherein Y is as defined in item 4, V is naratuximab and m is 2-10

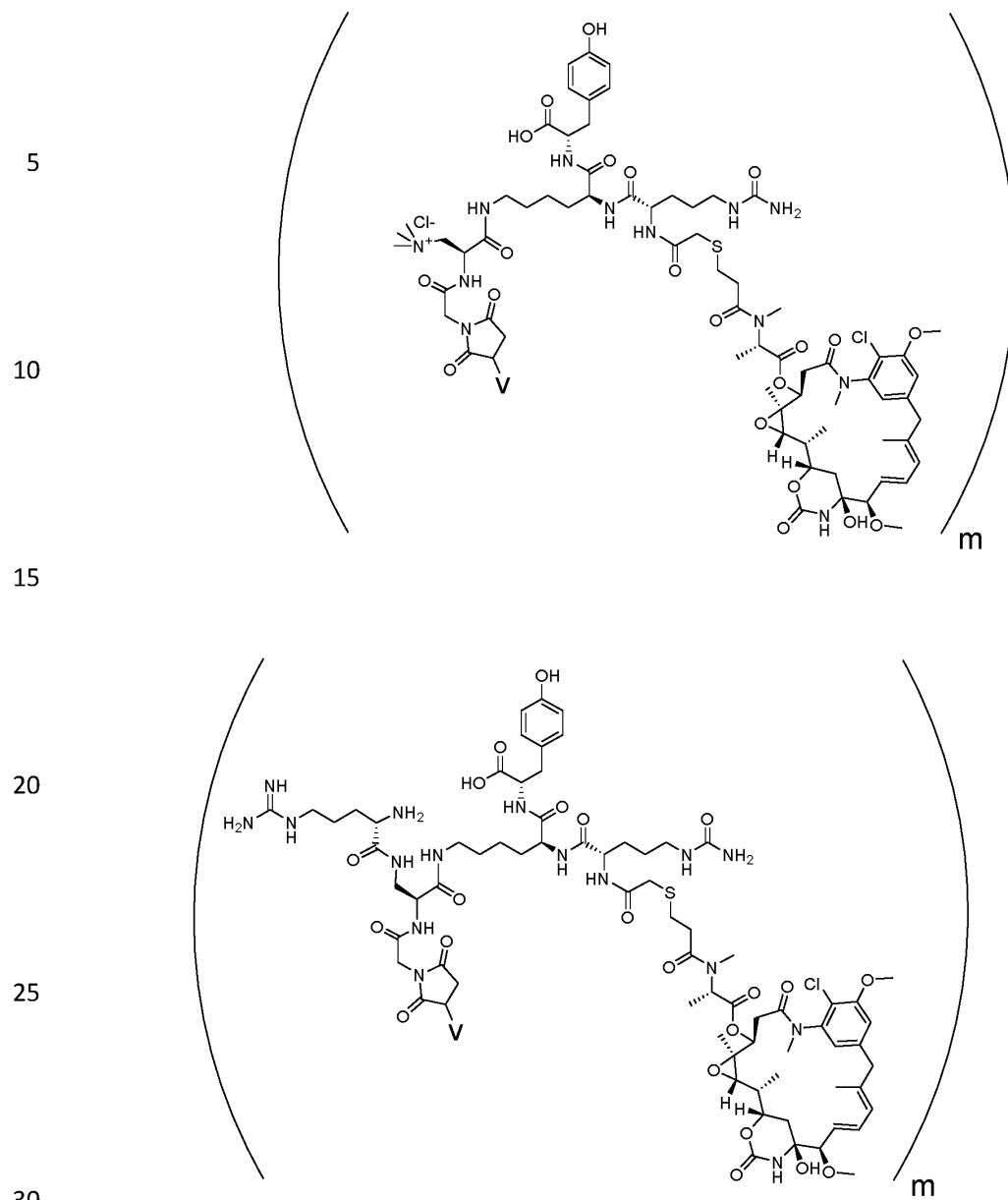
with the proviso that V is to be understood as being outside the parentheses, such that
5 m does not apply to V.

7. The combination of any one of items 1 to 5, wherein the conjugate compound of formula (I) is represented by one of the following formulae:





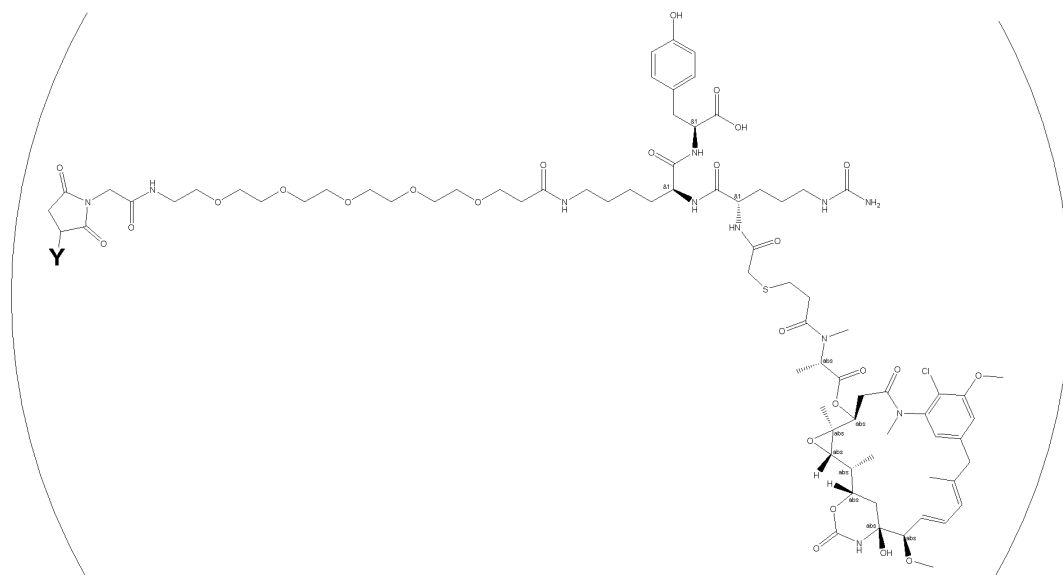




wherein V and m are as defined in any of the preceding claims and preferably V is naratuximab and m is 4 to 8,

with the proviso that V is to be understood as being outside the parentheses, such that m does not apply to V,

or the compound of formula (I) is a compound wherein V is as specified in claim 1 and preferably derived from naratuximab and the moiety -LL-D is represented by the following formula:



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8. The combination of item 1, wherein the conjugate compound of formula (I) contains a linker LL that is selected from N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP); N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB) or N-succinimidyl 4-(2-pyridyldithio)-2-sulfobutanoate (sulfo-SPDB); N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC); N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfoSMCC); N-succinimidyl-4-(iodoacetyl)-aminobenzoate (SIAB); and N-succinimidyl-[(N-maleimidopropionamido)-tetraethyleneglycol] ester (NHS-PEG4-maleimide) and preferably SMCC or NHS-PEG4-maleimide.

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9. The combination of item 1 or 8, wherein the conjugate compound of formula (I) is naratuximab emtansine (Debio1562, IMG529).

10. The combination of any one of items 1 to 9, wherein the combination comprises a BCL2 inhibitor or pharmaceutically acceptable salt, solvate or polymorph thereof and the BCL2 inhibitor is selected from venetoclax (ABT-199), gossypol, sonrotoclax (BGB 11417), navitoclax (ABT-263), obatoclax (GX15-070), obatoclax mesylate, ABT-737, AZD4320, AZD0466, TW-37, antimycin A, apogossypolone (Apo-G2), HA14-1, and chelerythrine.

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11. The combination of item 10, wherein the BCL2 inhibitor is a BCL2-selective inhibitor, preferably venetoclax, navitoclax or ABT-737.

12. The combination of any one of items 1 to 9, wherein combination comprises an inhibitor of the PI3K pathway or pharmaceutically acceptable salt, solvate or polymorph thereof, and the inhibitor of the PI3K pathway is a PI3K inhibitor that may for instance be selected from IPI-145, idelalisib (CAL-101), buparlisib (AN2025), roginolisib (MSC2360844), copanlisib (BAY 80-6946), IC-87114, PIK-93, pictilisib (GDC-0941), dactolisib (BEZ235), GSK1059615, BX-912, SF1126, pilarsilib (SAR245408), voxtalisib (SAR245409), BGT226, gedatolisib (PKI-587), NVPBE235, izorlisib (CH5132799), ZSTK474, sonolisib (PX-866), B591, TG100-115, RIDR-PI-103, alpelisib (BYL719), serabelisib (INK-117), GSK2636771, zandelisib (ME-401), AMG319, lisperlisib (YY-20394), parsaclisib (INCB050465), umbralisib (TGR-1202), PF-04691502, tenalisib (RP6530), taselisib (GDC-0032), AZD8186, AZD8835, duvelisib (IPI-145), leniolisib (CDZ173), eganelisib (IPI-549), apitolisib (GDC-0980), omipalisib (GSK2126458), samotolisib (LY3023414), bimiralisib (PQR309), paxalisib (GDC-0084), voxtalisib (XL765), SAR245409, VS-5584;

an Akt inhibitor that may for instance be selected from AT7867, MK-2206, perifosine (KRX-0401), triciribine, ipatasertib (RG7440), afuresertib (GSK2110183), uprosertib (GSK2141795), capivasertib (AZD5363), solenopsin, solenopsin analogues, HY-10249A, AT13148, KP372-1, GSK690693, erufosine, erucylphosphocholine, ilmofosine, edelfosine; or

an mTOR inhibitor that may for instance be selected from everolimus, temsirolimus, KU 0063794, AZD 8055, ridaforolimus (AP23573), umirolimus, zotarolimus, torin-1, sapanisertib (INK128), vistusertib (AZD2014), PP242, OSI-027, WYE354, WYR-125132, INK128/MLN-0128, deforolimus, onatasertib (CC-223), as well as the dual PI3K/mTOR inhibitors such as dactolisib, pilarlisib, voxtalisib, apitolisib, gedatolisib, BGT226, GSK2126458, PF-04691502, VS-5584, SF-1126; and preferably idelalisib, IPI-145, IC-87114, PIK-93, GDC-0941, GSK1059615, KU0063794, AZD8055, MK-2206 or triciribine.

13. The combination of any one of the preceding items for use in the treatment of cancer.

14. The combination for use according to item 13, wherein the cancer is DLBCL.

15. A conjugate compound of formula (I) or pharmaceutically acceptable salt, solvate or polymorph thereof, for use in a method of treating cancer, wherein the cancer is preferably DLBCL, and wherein the method comprises administering the conjugate compound of formula (I) or pharmaceutically acceptable salt, solvate or polymorph thereof in combination with a co-agent selected from a BCL2 inhibitor or pharmaceutically acceptable salt, solvate or polymorph thereof and a PI3K pathway inhibitor or pharmaceutically acceptable salt, solvate or polymorph thereof,

- wherein the conjugate compound of formula (I) is as specified in any one of items 1 to 9 and wherein the BCL2 inhibitor or pharmaceutically acceptable salt, solvate or polymorph thereof and a PI3K pathway inhibitor or pharmaceutically acceptable salt, solvate or polymorph thereof is preferably as specified in any one of items 10 to 12.

15 FIGURES

Figure 1 is a diagram showing the Loewe (A) and Bliss (B) synergy scores across a range of doses between N-DM1 and pictisilib in the assay described in Example 10.7.

- 20 Figure 2 is a diagram showing the Loewe (A) and Bliss (B) synergy scores across a range of doses between N-DM1 and navitoclax in the assay described in Example 10.7.

- Figure 3 shows the acquired resistance to naratuximab emtansine in SU-DHL-4 cell line using an MTT assay. Left panel: cell proliferation (viability %) of the SU-DHL-4 resistant cell line generated as described in Example 10.9, compared to that of the parental cell line. Right panel: bar plots correspond to IC₅₀ values of resistant and parental cell lines. *, P<0.05.

- 30 Figure 4 shows (A) the increased sensitivity to venetoclax and idelalisib of the SU-DHL-4 resistant cell line generated as described in Example 10.9, using an MTT assay. Left panels: cell proliferation (viability %) of the SU-DHL-4 resistant cell line generated as described in Example 10.9, compared to that of the parental cell line, upon increasing doses of venetoclax (top) or idelalisib (bottom). Right panel: bar plots correspond to IC₅₀ values of resistant and parental cell lines. *, P<0.05.

Figure 5 is an MTT plot showing the combination of naratuximab emtansine (Debio 1562) with venetoclax in both parental and resistant SU-DHL-4 cell lines, as described in Example 10.9. *, $P < 0.05$.

- 5 Figure 6 is an MTT plot showing the combination of naratuximab emtansine (Debio 1562) with idelalisib in both parental and resistant SU-DHL-4 cell lines, as described in Example 10.9. *, $P < 0.05$.

- Figure 7 shows MTT results obtained with idelalisib pretreatment as described in
10 Example 10.9. Bar plots correspond to IC50 values of resistant and parental cell lines.
*, $P < 0.05$.

DETAILED DESCRIPTION OF THE PRESENT INVENTION

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1. Definitions

- The expression “moiety derived from” as used herein characterizes a moiety that contains a group, which is identical to a specified compound except for the structural
20 modifications necessary for bonding the derived group to the remainder of the conjugate of the present invention. Depending on the functional groups available in the compound, bonding may be effected using one of the functional groups already present in the specified compound or it may be effected by incorporating a new functional group. By consequence, the specified compound can be used for bonding
25 in unmodified or in a modified form. That is, the specified compound can be unmodified (in its natural form) except for the replacement of a hydrogen atom by a covalent bond, or it can be chemically modified in order to incorporate one functional group (e.g., a group selected from hydroxyl, carboxyl, amino and thiol groups) allowing covalent attachment(s) to an adjacent group or moiety, e.g., to a divalent group (X) in formula
30 (I). If the specified compound is the drug compound DM1, said adjacent group or moiety may however remain attached to the drug after cleavage. The expression “moiety derived from DM1” or similar wordings, as used herein, thus refers to a moiety that differs from the unmodified (native) or modified (to incorporate one functional group) DM1 only by virtue of the covalent bond needed for bonding to the remainder
35 of the molecule, and is not meant to encompass any further group, for instance, a group or moiety that remains attached thereto after cleavage by Cathepsin B.

In an analogous manner, the term “derivative” is used to characterize moieties bonded to adjacent moieties, which moieties differ from the molecules from which they are derived only by the structural elements responsible for bonding to adjacent moieties. This may include covalent bonds formed by existing functional groups or covalent bonds and adjacent functional groups newly introduced for this purpose.

Likewise, unless specified otherwise or unless the context dictates otherwise, the expression “derived from” (such as in “derived from a compound”), when used in connection with other groups or moieties, is meant to describe a group or moiety, which is identical to the referenced compound or the like except for the structural modifications necessary for bonding the group or moiety to the one or more adjacent groups or moieties, typically by replacing a hydrogen atom or atomic group by a covalent bond (e.g. replacement of OH in a carboxyl group by a covalent bond upon amide bond formation with an amino group; further examples are given in the below table at the end of the section “Divalent group (X)”).

The term “native drug” refers to a compound, for which therapeutic efficacy has been established by *in vitro* and/or *in vivo* tests. In a preferred embodiment, the native drug is a compound for which therapeutic efficacy has been established by clinical trials. Most preferably, the native drug is a drug that is already commercially available. The type of therapeutic efficacy to be established and suitable tests to be applied depend of course on the type of medical indication to be treated.

When referring to specific classes of drug molecules, such as PI3K pathway inhibitor or BCL2 inhibitor, these terms are intended to have the meaning generally accepted in the field of medicine, as reflected, for instance, in the *Mosby's Medical Dictionary*, Mosby, Elsevier 10th ed. (2016), or in *Oxford Textbook of Oncology*, David J. Kerr, OUP Oxford 3rd ed. (2016).

Accordingly, the drug to be used in the conjugate compound of the present invention can be a native drug (e.g. a drug naturally containing one or more functional groups allowing covalent attachment to the conjugate), or can be a drug chemically modified to incorporate one functional group (e.g., a group selected from hydroxyl, carboxyl, amino and thiol groups) allowing covalent attachment(s) to an adjacent group or moiety) provided that the modified drug is pharmacologically active. Pharmacological activity in this connection means at least 20%, preferably at least 50%, more preferably at least 80% of the pharmacological activity of the native drug.

Furthermore, in those instances where the drug is released in a modified form insofar as a group or moiety remains attached thereto after cleavage by Cathepsin B, e.g., released as a moiety D-X or D-X-Dxx-Dyy, the modified drug can be referred to as an "intra-drug". In some instances, the remaining groups X, Dxx and Dyy may subsequently be removed via other mechanisms, e.g., by hydrolysis of an ester linkage that may be present between D and X. For those linkages that are not cleaved by other mechanisms, it is advantageous if the remaining modified drug D-X or D-X-Dxx-Dyy is pharmacologically active. Pharmacological activity in this connection means that the released modified drug, e.g., the moiety D-X or D-X-Dxx-Dyy, retains at least 20%, preferably at least 50%, more preferably at least 80% of the pharmacological activity of the native drug when released intracellularly by the ADC. To ensure realistic conditions, such a test for activity should not be made via cell cytotoxicity comparison of the released modified drug and the native drug because these conditions require entry of the modified drug into the cell, which may introduce a cell permeability bias. Differences in permeability between these two entities are not relevant here due to the intracellular release of the modified drug. It may be possible to compare activities of the modified drug and the native drug in a cell-free binding assay to determine K_i values (binding affinities) for the appropriate target receptor of the drug. If it is not possible to determine the K_i values, one may compare the IC_{50} of the cytotoxicity in HER2+ cell lines of two trastuzumab ADCs with exact same linker system (e.g. Val-Cit-PAB), one designed to release the modified drug and one to release the native drug.

The term "pharmaceutically acceptable salts" as used herein refers to derivatives of disclosed compounds (including the reactive conjugates) wherein the parent compound is modified by making acid or base salts thereof. The pharmaceutically acceptable salts include the non-toxic salts or the quaternary ammonium salts of the parent compound formed, for example, from non-toxic inorganic or organic acids or bases. Lists of suitable salts can be found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA, 1985, page 1418, S.M. Berge, L.M. Bighley, and D.C. Monkhouse, "Pharmaceutical Salts" J. Pharm. Sci. 1977, 66(1), 1–19; P. H. Stahl and C. G. Wermuth, editors, *Handbook of Pharmaceutical Salts: Properties, Selection and Use*, Weinheim/Zürich, Wiley-VCH, 2008 and in A.K. Bansal et al., *Pharmaceutical Technology*, 3(32), 2008. The pharmaceutical salts can be synthesized from the parent compound which contains a basic or acidic moiety by conventional chemical methods. For the reactive conjugates, this can be done before or after incorporating the drug moiety into the compound of the present invention. Unless the context dictates otherwise, all references to compounds (conjugates,

modified antibodies, etc.) of the invention are to be understood also as references to pharmaceutically acceptable salts of the respective compounds.

5 The term “backbone” refers to the connected link of atoms that span the length of a molecule. Accordingly, the expression “divalent group comprising X to Y backbone atoms” defines a group in which atoms, e.g., C, N, O, S, are covalently attached to each other to form a connected chain of atoms, said chain being covalently attached at its extremities to adjacent groups or moieties. The divalent group may include pendant atoms or groups that are attached to the backbone portion. Hydrogen atoms
10 that saturate valencies of the backbone atoms are not counted as backbone atoms. If cyclic groups are present, the backbone is identified as the shortest connection between the termini.

15 The term “functional group” refers to a group that is capable of bonding to another functional group by forming at least one covalent bond without need for breaking any C-C or C-H covalent bonds.

The expression “capable of being cleaved by Cathepsin B” characterizes any compound (or moiety that may be incorporated into a compound), which is cleaved
20 when being contacted with Cathepsin B (Cat B) under suitable conditions e.g., as set out in WO2019096867. In preferred embodiments, said cleavage is (a) fast and/or (b) cleavage is via the exopeptidase activity of Cat B. Said embodiment (b), relating to a compound or moiety that is “capable of being cleaved by the exopeptidase activity of Cat B”, is defined in more detail in the next paragraph. The above-mentioned “fast”
25 cleavage of embodiment (a) typically means for a compound of interest that the corresponding unconjugated compound (i.e. compound not having a vector group V and being quenched at the conjugation group, for example with cysteine being covalently attached to a maleimide conjugation group) has a cleavage rate $T_{1/2}$ of 25 min or less, preferably 20 min or less, more preferably 18 min or less, even more preferably 16 min or less and most preferably 14 min or less. There is no particular
30 lower limit. However, as the solubilizing group (PEG or the like) tends to reduce the cleavage rate, it is realistic to expect cleavage rates $T_{1/2}$ of 1 min or more, typically 2 min or more and even more typically 5 min or more.

35 The expression “capable of being cleaved by the exopeptidase activity of Cat B” as used herein indicates that the respective moiety of the compound, in particular the linker, e.g., C-terminal peptide unit, can be specifically recognized and cleaved by the exopeptidase (i.e., carboxydiptidase) of Cathepsin B. Said cleavage gives rise to the

rapid release of the drug (or a modified drug having group or moiety that remains attached thereto after cleavage by Cathepsin B, "intra-drug") into the target cell. The cleavage of a linker, e.g., a C-terminal peptide unit, via the exopeptidase activity of Cat B can be assessed by the *in vitro* enzymatic cleavage assay using recombinant human
5 Cat B and UHPLC-MS/MS analysis as described further below.

The term "solubilizing group" or "solubilizing moiety" as used herein refers to a hydrophilic group or moiety, which can enhance (improve) the water solubility of the moiety or compound to which it is attached. The solubilizing group can be, for example,
10 a polyalkylene oxide group, such as a polyethylene oxide (PEO) or a polypropylene oxide (PPO) group, a saccharide group or a moiety comprising one or more ionic or ionizable groups, i.e., functional groups which are charged (anionic or cationic) at physiological pH (7.4), such as moieties derived from amino acids, e.g., from Lys, Glu, Asp, His, Arg, diaminopropionic acid (Dap), diaminobutyric acid (Dab), 2-aminoadipic
15 acid (Aad), carnitine, Orn. Examples of ionic or ionizable groups include ammonium groups, guanidinium groups, sulfate groups, phosphate groups, phosphonate groups, and sulfonate groups. Examples of saccharide groups include monosaccharides, disaccharides and linear or branched oligosaccharides, in particular linear or branched oligosaccharides having 3 to 10 monosaccharide units being linked by glycosidic bond,
20 wherein each of the monosaccharide units in the monosaccharide, disaccharide and oligosaccharide is independently selected from glucose, fructose, mannose, ribose, and galactose. In the present invention, the term "solubilizing moiety" refers to a moiety derived from a compound comprising one or more, e.g., two, three or four, solubilizing groups. In further embodiments, the solubilizing moiety can consist of one or more
25 solubilizing groups, e.g., amino acids, PEO groups.

The term "polyalkylene oxide" (or "polyalkylene glycol", "polyoxyalkylene") as used herein refers to substances of the general structure $\text{HO}-(\text{X}-\text{O})_n-\text{H}$, wherein X represents an alkylene group having 2 or 3 carbons atoms, and n indicates the number
30 of repeating units, e.g., 6 to 200, 10 to 150, or 12 to 80 repeating units, such as 16 or 40 repeating units, e.g., 17, 18, 20 or 24 PEO repeating units. Thus, the term "polyalkylene oxide group" is to be understood as a divalent group of formula $^*-\text{O}-(\text{X}-\text{O})_n-^{**}$, wherein X and n are as defined above, and * and ** indicate covalent attachment to adjacent moieties. In some embodiments, the term "polyalkylene oxide"
35 can refer to polyethylene oxide (or polyethylene glycol, C₂-polyalkylene oxide), or polypropylene oxide (or polypropylene glycol, C₃-polyalkylene oxide). It is also possible to provide a polyalkylene oxide group, in which two or more different alkylene groups, as defined above, are arranged in a random or block-wise manner.

The term "peptide" as used herein refers to a compound comprising a continuous sequence of at least two amino acids linked to each other via peptide linkages. The terms "dipeptide", "tripeptide" and "tetrapeptide" respectively refer to a compound comprising a continuous sequence of two, three and four amino acids linked to each other via peptide linkages. The term "peptide linkage" in this connection is meant to encompass (backbone) amide bonds as well as modified linkages, which can be obtained if non-natural amino acids are introduced in the peptidic sequence. In this case, the modified linkage replaces the (backbone) amide bond which is formed in the continuous peptide sequence by reacting the amino group and the carboxyl group of two amino acid residues. For instance, the modified linkage may be an ester, a thioester, a carbamide, a thiocarbamide or a triazole linkage. Preferably, the amino acids forming the continuous peptide sequence are linked to each other via backbone amide bonds. The peptide may be linear or branched. In preferred aspects, the peptide is a linear di-, tri-, tetra-peptide, more preferably a linear tri- or tetra-peptide.

The term "amino acid" as used herein refers to a compound that contains or is derived from a compound containing at least one amino group and at least one acidic group, preferably a carboxyl group. The distance between amino group and acidic group is not particularly limited. α -, β -, and γ -amino acids are suitable but α -amino acids and especially α -amino carboxylic acids are particularly preferred. The term "amino acid" encompasses both naturally occurring amino acids such as the naturally occurring proteinogenic amino acids, as well as synthetic amino acids that are not found in nature. In the following, a reference to amino acids may be made by means of the 3-letter amino acid code (Arg, Phe, Ala, Cys, Gly, Gln, etc.), or by means of the 1-letter amino acid code (R, F, A, C, G, Q, etc.).

Hereinafter, amino acid sequences are written from the N-terminus to the C-terminus (left to right). Unless specified otherwise or dictated otherwise by the context, all connections between adjacent amino acid groups are formed by peptide (amide) bonds.

The expression "amino acid in the (D) configuration" as used herein refers to the (D)-isomer of any naturally occurring or synthetic amino acid. This applies to α -amino acids as well as to β - and γ -amino acids. The expression "amino acid in the (D) configuration" as used herein is not meant to encompass non-chiral amino acids such as glycine or other non-chiral amino acids such as aminoisobutyric acid.

The expression "side chain of an amino acid" as used herein may refer to a moiety attached to the α -carbon of an amino acid. For example, the side chain of Ala is methyl, the side chain of Phe is phenylmethyl, the side chain of Cys is thiomethyl, the side chain of Tyr is 4-hydroxyphenylmethyl, etc. Both naturally occurring side chains and
5 non-naturally occurring side chains are included by this definition.

The term "trifunctional" as used herein refers to a compound or moiety having three functional groups that can form or have formed three covalent bonds to adjacent moieties. Thus, the term "trifunctional amino acid" refers to a compound that contains
10 or is derived from a compound containing at least an amino group, an acid group (e.g. a carboxyl group) and another functional group such as an amino group or a carboxyl group. Non-limiting examples of trifunctional amino acids include Ser, Cys, Tyr, N- ϵ -propargyloxycarbonyl-L-Lysine (Lys(Poc)), Asp, Glu, Orn, Lys, Dab and Dap.

15 The term "C-terminal" as used herein refers to the C-terminal end of the amino acid (peptide) chain. Binding to the "C-terminus" means that a covalent bond is formed between the acid group in the main chain (backbone) of the amino acid residue and the binding partner. For instance, binding of group "X" to the C-terminus of amino acid residue "Axx" yields an ester or amide-type structural element $\text{*}-\text{C}(\text{O})-\text{X}$, wherein the
20 carbonyl group is derived from the acid group of Axx and (*) indicates attachment to main chain. The term "C-terminal peptide unit" is used herein to characterize a peptide sequence of 2, 3 or 4 amino acids wherein the C-terminal amino acid forms the C-terminus of the peptide sequence.

25 The term "N-terminal" as used herein refers to the N-terminal end of the amino acid (peptide) chain. Binding to the "N-terminus" means that a covalent bond is formed between the amino group in the main chain (backbone) of the amino acid residue and the binding partner (which replaces one hydrogen atom). For instance, binding of group "X" to the N-terminus of amino acid residue "Axx" yields a structural element $\text{X}-\text{NH}-\text{*}$,
30 wherein the amino group is derived from Axx and (*) indicates attachment to main chain.

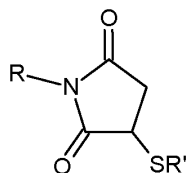
The term "hydrophobic" is used herein to characterize compounds, groups or moieties, which lack affinity for water. For instance, the term "amino acid with hydrophobic side
35 chain" is used to characterize amino acids with a hydrophobic or partially hydrophobic aliphatic side chain or amino acids with aromatic side chain such as Phe, Leu, Ile, Val, Tyr, Trp, Ala. Of course, any other amino acid exhibiting the same or a higher degree of hydrophobicity should also be treated as hydrophobic in the sense of the present

invention. A comparison of the degree of hydrophobicity can be done by determining the n-octanol/water partition coefficient (at 25°C and pH 7): if the ratio of concentrations in n-octanol/water for another amino acid is equal or higher than that of one or more of the amino acids Phe, Leu, Ile, Val, Tyr, Trp, Ala, such other amino acid is to be treated
5 as a hydrophobic amino acid.

The term “amino acid with a basic side chain” is used herein to characterize natural or unnatural amino acids wherein the side chain contains one or more ionizable groups having a pKa value equal to or greater than 6. Examples of natural amino acids with a
10 basic side chain include Arg (guanidino group, pKa=12.5), Lys (amino group, pKa=10.5), His (imidazole group, pKa=6). Examples of unnatural amino acids with a basic side chain include citrulline (Cit), ornithine (Orn), 2,3-diamino-propionic acid (Dap), 2,4-diamino-butyric acid (Dab).

15 The term “alkyl group” as used herein refers to a linear or branched hydrocarbon group having from 1 to 20 carbon atoms, preferably from 1 to 5 carbon atoms, more preferably a methyl or an ethyl group, or to a cycloalkyl group having from 3 to 20 carbon atoms, preferably from 5 to 8 carbon atoms. The term “cycloalkyl group” as
20 used herein refers to a cycloalkyl group having from 3 to 20 carbon atoms, preferably from 5 to 8 carbon atoms. The cycloalkyl group may consist of a single ring, but it may also be formed by two or more condensed rings.

The term “divalent maleimide derivative” (or, e.g., “divalent group derived from a compound selected from maleimide...”) as used herein refers to a divalent moiety
25 derived from maleimide (e.g., a succinimide moiety), in which the double bond is hydrogenated and two hydrogen atoms are replaced by two covalent bonds allowing attachment to adjacent moieties. For example, the divalent maleimide derivative may have the following structure (wherein R and R' represent adjacent moieties to which
30 said maleimide derivative is attached):



Said moiety contains a chiral carbon atom (i.e. the atom carrying the sulfur atom). Unless specified otherwise, references to a divalent maleimide derivative are to be

understood as references to the pure stereoisomers as well as any mixture thereof and especially the racemic mixture thereof.

5 The term “divalent maleimide derivative” or “divalent group derived from a compound selected from maleimides” is further to be understood as encompassing any derivative of maleimide (as described above) additionally being substituted at other positions than positions 2 and 3, as well as opened hydrolyzed maleimide derivatives.

10 A divalent maleimide-type disulfide bridge (e.g. a divalent group of formula $-S-X^2-S-/-S-X^3-S-$ wherein X^2/X^3 represents a divalent group derived from maleimide) can be obtained by side-chain-to-side-chain cyclization in the presence of e.g. 2,3-dibromomaleimide or another suitable reagent as described by Kuan et al. in *Chem. Eur. J.* 2016, 22, 17112-17129.

15 In the context of the invention, an “opened hydrolyzed maleimide derivative” refers to a divalent moiety derived from maleimide (as described above) wherein the maleimide ring has been opened by hydrolysis. For instance, the hydrolysis of the divalent maleimide derivative $R-X-S-R'$ (wherein X represents an unsubstituted divalent group derived from maleimide and R/R' represent adjacent groups or moieties; in X the
20 double bond of maleimide is no longer present) leads to an opened hydrolyzed maleimide derivative of formula $R-NH-C(=O)-CH(S-R')-CH_2-COOH$, or $R-NH-C(=O)-CH_2-CH(S-R')-COOH$, or a mixture thereof. The ring hydrolysis can be performed, for example, under basic conditions. The following conditions are especially suitable: at the end of a cysteine-maleimide conjugation reaction (e.g., after the reaction of a
25 maleimide moiety (Y') with the side chain of a cysteine residue contained in a molecule capable of interacting with a target cell (V')), pH is adjusted to pH 8 by adding 10x pH 8 DPPS (0.2 to 0.5 reaction volume) and excess reactive drug linker and reducing agent (TCEP) are removed via gel filtration using suitable columns for gel filtration (e.g., PF column, elution with pH 8 buffer), the eluent is then stirred overnight for 16h
30 to complete the opening before final buffer exchange with DPBS into an Amicon concentrating unit. Unless specified otherwise, any reference to an “opened hydrolyzed maleimide derivative” is to be understood as a reference to one of these structures alone or any mixture of these structures. Moreover, the carbon carrying the sulfur atom is chiral. Unless specified otherwise, any reference to an “opened
35 hydrolyzed maleimide derivative” is to be understood as a reference to the pure stereoisomers as well as any mixture thereof and especially the racemic mixture thereof.

Furthermore, in the context of the invention, a “maleimide attachment” refers to a divalent moiety derived from maleimide as described above which contains two covalent bonds allowing attachment to adjacent groups or moieties. For example, in the maleimide derivative of the formula $R-X-S-R'$, where R/R' represent adjacent groups or moieties, X represents the maleimide attachment (a divalent group derived from maleimide in which the double bond of maleimide is no longer present). Thus, the term “maleimide attachment” is synonymous with “maleimide derivative attachment”.

Similarly, in the context of the invention, an “opened hydrolyzed attachment” refers to a divalent moiety derived from maleimide as described above which contains two covalent bonds allowing attachment to adjacent groups or moieties. For example, in the opened hydrolyzed maleimide derivative of the formula $R-X-S-R'$, where R/R' represent adjacent groups or moieties, X represents the opened hydrolyzed maleimide attachment. Thus, the term “opened hydrolyzed attachment” is synonymous with “opened hydrolyzed derivative attachment”.

References to “a divalent group derived from a compound selected from... carbonyl-containing compounds” are meant to characterize divalent groups $-C(=O)-X-$ with X representing O, S or NH, resulting from reacting (an activated) carbonyl with a nucleophilic group such as formation of an amide group, ester group, thioester group. In the context of group Y, the divalent group may be formed by reacting a carbonyl-containing group (e.g. $-C(=O)-Cl$) attached to V with a nucleophilic group (e.g. $-NH_2$) attached to T, or vice versa.

In the above divalent groups, the term “derivative thereof” means that any hydrogen atom may be replaced by a substituent, as defined hereinbelow, as long as the substitution does not interfere with divalent group formation.

The expression “capable of interacting with a target cell” as used herein indicates that the vector group can bind to, complex with, or react with a moiety, e.g. a protein or receptor, that is exposed on the surface of a target cell. Said interaction may give rise to a targeting effect (i.e. to a local increase of the concentration of the vector-carrying compound in the vicinity of the target cell) and/or it may cause internalization of the vector-carrying compound of the present invention into the target cell.

The term “antibody” (also synonymously called “immunoglobulin” (Ig)) as used herein covers monoclonal antibodies, polyclonal antibodies, dimers, multimers, multi-specific antibodies (e.g. bispecific antibodies), veneered antibodies, and small immune

proteins. An antibody is a protein generated by the immune system that is capable of recognizing and binding to a specific antigen. A target antigen generally has numerous binding sites, also called epitopes, recognized by complementary-determining regions on multiple antibodies. Each antibody that specifically binds to a different epitope has a different structure. Thus, one antigen may have more than one corresponding antibody. An antibody includes a full-length immunoglobulin molecule or an immunologically active portion of a full-length immunoglobulin molecule, i.e. a molecule that contains an antigen-binding site that immuno-specifically binds an antigen of a target of interest or part thereof. The antibodies may be IgG e.g. IgG1, IgG2, IgG3, IgG4. Preferably, the antibody is an IgG protein and more preferably an IgG1, IgG2 or IgG4 protein. Most preferably the antibody is an IgG1 protein. The antibody can be human or derived from other species. Preferably the antibody is a human antibody.

The term "monoclonal antibodies" as used herein characterizes antibodies that are identical because they are produced by one type of immune cell and are all clones of a single parent cell.

The term "antibody fragment" as used herein refers to a molecule comprising at least one polypeptide chain derived from an antibody that is not full length.

The term "Fc-fusion protein" as used herein refers to a protein comprising at least an Fc-containing antibody fragment - i.e. an immunoglobulin-derived moiety comprising at least one Fc region - and a moiety derived from a second, non-immunoglobulin protein. The Fc-containing antibody fragment forms part of the Fc-fusion protein and therefore is incorporated into the Fc-fusion protein. The Fc-containing antibody fragment can be derived from an antibody as described hereinabove, in particular from IgG e.g. IgG1, IgG2, IgG3, IgG4. Preferably, the Fc-containing moiety is derived from an IgG1 protein, more preferably from a human IgG1 protein. The non-Ig protein can be a therapeutic protein, for instance a therapeutic protein derived from erythropoietin (EPO), thrombopoietin (THPO) such as THPO-binding peptide, growth hormone, interferon (IFN) such as IFN α , IFN β or IFN γ , platelet-derived growth factor (PDGF), interleukin (IL) such as IL1 α or IL1 β , transforming growth factor (TGF) such as TGF α or TGF β , or tumor necrosis factor (TNF) such as TNF α or TNF β , or a therapeutic protein derived from a receptor, in particular from a ligand-binding fragment of the extracellular domain of a receptor, for instance derived from cluster of differentiation 2 (CD2), CD4, CD8, CD11, CD14, CD18, CD20, CD22, CD23, CD25, CD33, CD40, CD44, CD52, CD58 (LFA3), CD80, CD86, CD147, CD164, IL2 receptor, IL4 receptor, IL6 receptor, IL12 receptor, epidermal growth factor (EGF) receptor, vascular

endothelial growth factor (VEGF) receptor, epithelial cell adhesion molecule (EpCAM), or cytotoxic T-lymphocyte-associated protein 4 (CTLA4).

The term "cancer" as used herein means the physiological condition in mammals that is characterized by unregulated cell growth. A tumor comprises one or more cancer cells.

The term "DLBCL" as used herein stands for diffuse large B-cell lymphoma, which is the most common form of non-Hodkin lymphoma among adults. It includes the "activated B cell" (ABC) subtype and the "germinal B cell" (GBC) subtype. Further information on DLBCL is described in "Diffuse large B-cell lymphoma: 2019 update on diagnosis, risk stratification, and treatment" by Y.Liu and S. K. Barta, Am. J. Hematol. 2019; 94, 604-616 and references cited therein, which is incorporated herein by reference.

The term "drug-antibody-ratio" (or "DAR") as used herein refers to the average number of drug molecule(s) attached to one (e.g., antibody) moiety (V). The DAR is sometimes referred to in the art as "drug load", or "drug loading". The DAR in the compound of the present invention may be calculated by multiplying n by m in Formula (1). If n=1, then m in Formula (1) represents the DAR. However, it is also understood the DAR will often be an average value when used to describe a sample containing many molecules, due to some degree of inhomogeneity, typically associated with the conjugation step. The average DAR, for instance, may be in the range of about 1 to about 10, and may be about 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10. In some aspects, the DAR may be from about 3 and about 8, and may be typically about 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5 or 9. In some aspects, the DAR may be about 4. In some aspects, the DAR may be about 8. In some aspects, a DAR of 'about n' means that the measured value for DAR is within $\pm 20\%$ of n (e.g., between 80% of n and 120% of n).

The term „emtansine“ as used herein refers to the complex formed by covalent attachment of the non-reducible linker N-succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate ("SMCC", designated as "MCC" after conjugation to e.g. an antibody) to the antimitotic agent mertansine (DM1). Emtansine is used in the known antibody-drug-conjugate trastuzumab emtansine (Kadcyla®).

The concept of "BCL2 inhibition" and "BCL2 inhibitors" is well known in the art. It is for instance discussed in the review article by S. Hafezi and M. Rahmani in Cancers 2021,

13, 1292 and literature cited therein. BCL2 inhibitors are typically characterized by an IC50 value ranging from 100nM to 1µM in at least one DLBCL cell model and/or an IC50 value ranging from 0.05 nM to 300 nM in a cell-free assay.

- 5 The concept of “inhibition of the PI3K pathway” and “PI3K pathway inhibitors” including also the concepts of inhibition of the proteins involved in this pathway, i.e., “PI3K inhibition”, “Akt inhibition” and “mTOR inhibition” is well known in the art. It is for instance discussed in the review article by A. Majchrzak et al. in *Molecules* 2014, 19, 14304-14315 and literature cited therein or Mishra R, Patel H, Alanazi S, Kilroy MK, Garrett JT. PI3K Inhibitors in Cancer: Clinical Implications and Adverse Effects. *Int J Mol Sci.* 2021 Mar 27;22(7):3464. doi: 10.3390/ijms22073464 and references cited therein. PI3K pathway inhibitors are typically characterized by an IC50 value ranging from 500nM to 10 µM in at least one DLBCL cell model and/or an IC50 value ranging from 0.05 nM to 400 nM in a cell-free assay in relation to at least one member of the PI3K pathway (i.e., PI3K, Akt, mTOR).

The term “patient” as used herein refers to a subject to which a combination of the present invention, i.e., a ligand-drug-conjugate and the co-agent, is administered. In the context of the present invention, the patient is a mammal, and preferably a human (male or female). In particular, the patient is a cancer patient and especially a DLBCL patient.

“Combination”, as used herein, refers to the presence of the combination partners, comprising at least the conjugate compound and BCL2 inhibitor or PI3K pathway inhibitor. The combination partners may be provided in the same pharmaceutical composition, or they may be provided separately, for instance in two separate pharmaceutical compositions. Likewise, any references to administration “in combination” are to be understood as administration of the combination partners to the same patient such that the combination partners may be administered together or separately from each other. If they are administered separately from each other, administration includes simultaneous (concurrent) and consecutive administration in any order. For instance, the conjugate compound and BCL2 inhibitor or PI3K pathway inhibitor may be administered in combination by administering the conjugate compound first and the BCL2 inhibitor or PI3K pathway inhibitor later, or vice versa.

As used herein, terms such as “treating” or “treatment” or “to treat” or “alleviating” or “to alleviate” refer to therapeutic measures that cure, slow down, lessen symptoms of, and/or halt or reverse progression or severity of a diagnosed pathologic condition,

disorder or disease. Thus, those in need of treatment include those already diagnosed with or suspected of having the disorder. In certain aspects, a subject is successfully "treated" for cancer according to the methods of the present invention if the patient shows one or more of the following: a reduction in the number of or complete absence of cancer cells; a reduction in the tumor size or burden; inhibition of or an absence of cancer cell infiltration into peripheral organs; inhibition of or an absence of tumor metastasis; inhibition of or an absence of tumor growth; relief of one or more symptoms associated with the specific cancer; reduced morbidity and mortality; improvement in quality of life; reduction in tumorigenicity, tumorigenic frequency, or tumorigenic capacity, of a tumor; reduction in the number or frequency of cancer stem cells in a tumor; differentiation of tumorigenic cells to a non-tumorigenic state; as well as increased chances to have a complete response (CR), a partial response (PR), increased chances to have the disease under control (e.g. CR, PR, stable disease SD), to live longer without progression, and without disease, to live longer, decreased chances to have a progressive disease (PD) and to increase the time until progression. Collectively for groups or populations of patients, successful treatment may result in endpoints such as increased Overall Response Rate (ORR), Best Overall Response (BOR), Duration of Response (DOR), Disease Control Rate (DCR), progression-free survival (PFS), overall survival (OS), time to progression (TTP) or any combination thereof.

As used herein when referring to cancer or to a patient suffering therefrom, the terms "relapse", "to relapse", "recurrence", "to recur" mean a worsening of the disease and/or of the signs and symptoms of the disease after a period of improvement, stabilization or disease absence.

As used herein when referring to cancer or to a patient suffering therefrom, the terms "progression" or "to progress" means when the cancer becomes worse, either due to existing lesions that are growing and/or due to appearance of new lesions.

As used herein, the term "therapeutically effective amount" refers to an amount of a drug effective to "treat" a disease or disorder in a subject or patient. In the case of cancer, the therapeutically effective amount of the drug can reduce the number of cancer cells; reduce the tumor size or burden; inhibit (i.e., slow to some extent and in a certain aspect, stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and in a certain aspect, stop) tumor metastasis; inhibit, to some extent, tumor growth; relieve to some extent one or more of the symptoms associated with the cancer; and/or result in a favorable response such as increased Overall Response

Rate (ORR), Best Overall Response (BOR), Duration of Response (DOR), Disease Control Rate (DCR), progression-free survival (PFS), overall survival (OS), complete response (CR) rate, partial response (PR) rate, or, in some cases, stable disease (SD) rate, a decrease in progressive disease (PD), an increased time to tumor progression (TTP) or any combination thereof. See the definition herein of "treating".

In some aspects, the term "therapeutically effective amount" of a given drug when used in monotherapy, such as a "conjugate compound therapeutically effective amount", a "BCL2 inhibitor therapeutically effective amount", a "PI3K pathway inhibitor therapeutically effective amount" or a "combination therapeutically effective amount", refers to an amount of a drug or combination of drugs effective to "treat" a disease or disorder in a subject or patient, whilst keeping an acceptable safety profile. In some aspects, when administering a conjugate compound with a BCL2 inhibitor or PI3K pathway inhibitor in combination, their respective therapeutically effective amounts may be referred to as the "combination therapeutically effective amount". The therapeutically effective amounts of the conjugate compound or the BCL2 inhibitor or PI3K pathway inhibitor, when administered as monotherapy, may be different from the "combination therapeutically effective amount". In the treatment of cancer, the therapeutically effective amount of the combination can reduce the number of cancer cells; reduce the tumor size or burden; inhibit (i.e., slow to some extent and in a certain aspect, stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and in a certain aspect, stop) tumor metastasis; inhibit, to some extent, tumor growth; relieve to some extent one or more of the symptoms associated with the cancer; and/or result in a favorable response such as increased Overall Response Rate (ORR), Best Overall Response (BOR), Duration of Response (DOR), Disease Control Rate (DCR), progression-free survival (PFS), overall survival (OS), complete response (CR) rate, partial response (PR) rate, or, in some cases, stable disease (SD) rate, a decrease in progressive disease (PD), an increased time to tumor progression (TTP) or any combination thereof. See the definition herein of "treating".

As used herein, "Overall Survival" (OS) in a clinical trial refers to the time from patient enrollment, first treatment administration or randomization to death from any cause or censored at the date last known alive. Improvement in OS includes a prolongation in life expectancy as compared to naive or untreated individuals or patients. Overall survival refers to the situation wherein a patient remains alive for a defined period of time, such as one year, five years, etc., e.g., from the time of randomization or first treatment.

As used herein, the term "pharmaceutical formulation" or "pharmaceutical composition" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

- 5 As used herein, the expression "oral dosage form" refers to any form of a pharmaceutical composition that is suitable for oral administration.

It is to be understood that any reference to a conjugate compound, a BCL2 inhibitor or a PI3K pathway inhibitor also encompasses the pharmaceutically acceptable salt thereof, even if not explicitly mentioned as such.

- 10 Information introduced in the present disclosure by the wording „in some aspects“, „in specific aspects“, or the like, is to be understood such that it can be freely combined with any other information provided elsewhere in the present disclosure, irrespective whether this is also introduced by the wording „in some aspects“, „in specific aspects“, or any other wording. An exception to this general rule applies however for those
- 15 instances, where the information to be combined is no consistent or even contradictory or mutually exclusive, so that the resulting combined information would be inconsistent, unclear and/or not technically meaningful. Likewise, where the present description refers to "preferred" embodiments/features, combinations of these "preferred" embodiments/features shall also be deemed as disclosed as long as this combination
- 20 of "preferred" embodiments/features is technically meaningful.

Hereinafter, in the present description of the invention and the claims, the use of the terms "containing" and "comprising" is to be understood such that additional unmentioned elements may be present in addition to the mentioned elements.

- 25 However, these terms should also be understood as disclosing, as a more restricted embodiment, the term "consisting of" as well, such that no additional unmentioned elements may be present, as long as this is technically meaningful. For instance, the expression "divalent carbonyl-containing group" includes as a preferred embodiment a divalent group consisting of carbonyl (-CO-). Moreover, the expression "at least one of
- 30 X and Y" is to be understood broadly as disclosing one or both of X and Y, i.e. as being equivalent to the expression "at least one selected from the group of X and Y".

- Unless specified otherwise or the context dictates otherwise, references to groups being "substituted" or "optionally substituted" are to be understood as references to the
- 35 presence (or optional presence, as the case may be) of at least one substituent

selected from F, Cl, Br, I, CN, NO₂, NH₂, NH-C₁₋₆-alkyl, N(C₁₋₆-alkyl)₂, -X-C₁₋₆-alkyl, -X-C₂₋₆-alkenyl, -X-C₂₋₆-alkynyl, -X-C₆₋₁₄-aryl, -X-(5-14-membered heteroalkyl with 1-3 heteroatoms selected from N, O, S), wherein X represents a single bond, -(CH₂)-, -O-, -S-, -S(O)-, -S(O)₂-, -NH-, -CO-, or any combination thereof including, for instance, -C(O)-NH-, -NH-C(O)-. The number of substituents is not particularly limited and may range from 1 to the maximum number of valences that can be saturated with substituents. It is typically 1, 2 or 3 and usually 1 or 2, most typically 1.

Unless specified otherwise, all valencies of the individual atoms of the compounds or moieties described herein are saturated. In particular, they are saturated by the indicated binding partners. If no binding partner or a too small number of binding partners is indicated, the remaining valencies of the respective atom are saturated by a corresponding number of hydrogen atoms.

Unless specified otherwise, chiral compounds and moieties may be present in the form of a pure stereoisomer or in the form of a mixture of stereoisomers, including the 50:50 racemate. In the context of the present invention, references to specific stereoisomers are to be understood as references to compounds or moieties, wherein the designated stereoisomer is present in at least 90% enantiomeric excess (ee), more preferably at least 95 %ee and most preferably 100 %ee, wherein %ee is defined as $(|R-S|)/(R+S)*100\%$ with R and S representing the amount of moles of the respective enantiomers.

Unless the context dictates otherwise, and/or alternative meanings are explicitly provided herein, all terms are intended to have meanings generally accepted in the art, as reflected by IUPAC Gold Book (status of 1st Aug. 2020), or the Dictionary of Chemistry, Oxford, 6th Ed.

As used herein, including in the appended claims, the singular forms of words such as "a", "an", and "the", include their corresponding plural references unless the context clearly indicates otherwise.

The term "and/or" as used herein in a phrase such as "A and/or B" herein is intended to include both "A and B," "A or B," "A," and "B." Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following aspects: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).

Administration at “approximately the same time” refers to administration at a time that is the same as the time of administration of the reference day or a time that deviates from the time of administration of the reference date by no more than about one hour.

5 **2. Overview**

The present invention relates to a combination of a conjugate compound and a BCL2 inhibitor or PI3K pathway inhibitor.

- 10 The present invention further relates to a combination of a conjugate compound and a BCL2 inhibitor or PI3K pathway inhibitor for use as a medicament. The present invention also relates to a method of using a combination of a conjugate compound and a BCL2 inhibitor or PI3K pathway inhibitor as a medicament. The use or method can comprise administering a therapeutically effective amount of the conjugate compound, and a therapeutically effective amount of the BCL2 inhibitor or PI3K pathway inhibitor, or a therapeutically effective amount of the combination of the conjugate compound and the BCL2 inhibitor or PI3K pathway inhibitor.

- 20 The present invention relates to a combination of a conjugate compound or a pharmaceutically acceptable salt thereof, and a BCL2 inhibitor or PI3K pathway inhibitor or a pharmaceutically acceptable salt thereof for use in, or for use in the preparation of a medicament for, treating cancer and especially DLBCL in a patient in need thereof.

- 25 Any disclosure relating to a conjugate compound herein is understood to be combined with any disclosure relating to a BCL2 inhibitor or PI3K pathway inhibitor herein.

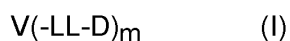
In some aspects of the present invention, the conjugate compound is a compound of formula (I) or a pharmaceutically acceptable salt thereof.

- 30 In some aspects of the present invention, the conjugate compound is a compound of formula (I) or a pharmaceutically acceptable salt thereof, and venetoclax as the BCL2 inhibitor or idelalisib as the PI3K pathway inhibitor or a pharmaceutically acceptable salt thereof.

35

3. Conjugate compound

The conjugate compound to be used in the present invention is a compound, i.e., a ligand-drug-conjugate (LDC), as represented by the general formula (I):



wherein V represents an anti-CD37 antibody, a fragment or derivative thereof or Fc-fusion protein containing an anti-CD37 fragment and preferably naratuximab, a fragment or derivative thereof or Fc-fusion protein containing a naratuximab fragment,

LL represents a linker covalently bonded to V and D,

D represents a moiety derived from a drug selected from maytansine, mertansine (DM1) and raptansine (DM4), preferably DM1 or DM4 and more preferably DM1; and

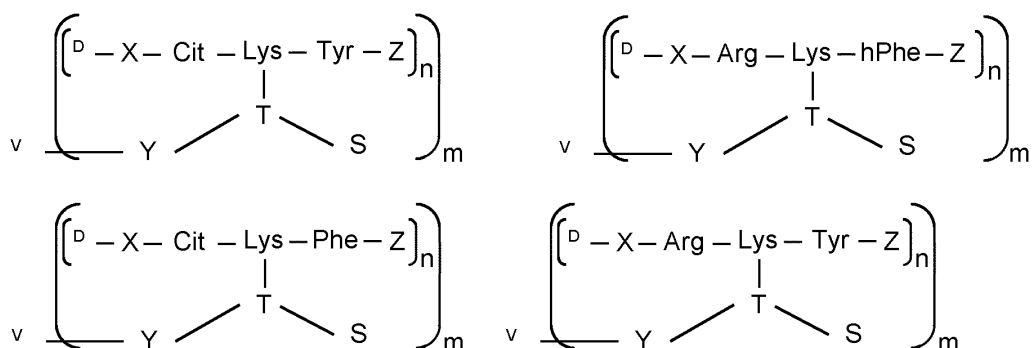
m is an integer of 1 to 12, preferably 2 to 10 and more preferably 4 to 8.

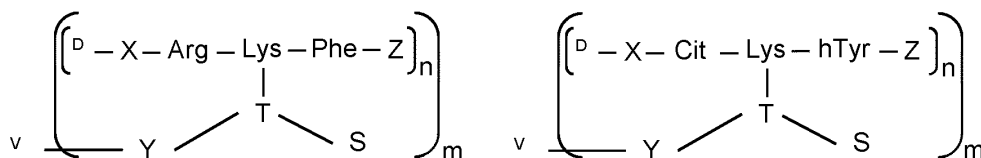
3.1 Linker

The conjugate compound for use in the present invention may contain a cleavable or non-cleavable linker.

Cleavable linkers are for instance described hereinbelow with respect to linker LL of formula (I).

The cleavable linker LL of formula (I) may be selected from the following formulae: represented by one of the following formulae:





5

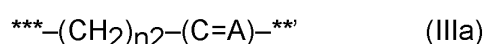
wherein ^D and ^V represent the positions of covalent attachment to D and V, respectively. The meanings of the further variable groups S, T, X, Y, Z, n and m are described in detail below. In a preferred embodiment, the meanings are as follows:

10

D represents a moiety derived from a drug selected from maytansine, mertansine (DM1) and ravidansine (DM4), preferably DM1 or DM4 and more preferably DM1;

15

X is a group of formula (IIIa),



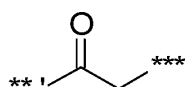
wherein n₂ is 1 or 2,

A is selected from O and S, preferably O;

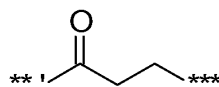
20

*** represents covalent attachment to D; and

** represents covalent attachment to the adjacent amino acid Arg, Cit or Phe; preferably a group represented by formula (IVc) or (IVd)



(IVc)

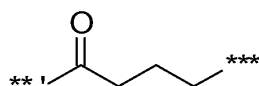


(IVd)

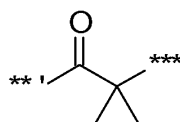
25

or

X is a group represented by formula (IVe) or (IVf)



(IVe)



(IVf)

30

*** represents covalent attachment to D;

*** represents covalent attachment to the adjacent amino acid Arg, Cit or Phe;

5 Y is a group derived from a compound selected from maleimides, triazoles, hydrazones, carbonyl-containing compounds and derivatives thereof, preferably from maleimides and derivatives thereof such as opened hydrolyzed maleimide derivatives, and more preferably derived from an opened hydrolyzed maleimide;

10 T is derived from an amino acid selected from N-ε-propargyloxycarbonyl-L-Lysine (Lys(Poc)), Asp, Glu, Orn, Lys, Dab and Dap, preferably derived from Lys(Poc), Glu, Orn or Lys, more preferably derived from Lys;

S is a moiety of formula (V)

15
$$****-X^1-(CH_2CH_2O)_{n3}-X^2 \quad (V)$$

wherein

n3 is an integer of 6 to 200, preferably 10 to 150, more preferably 12 to 80;

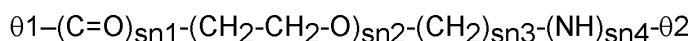
20 **** indicates covalent attachment to T, preferably the side chain of T;

25 X¹ is selected from a single covalent bond, -(C=O)-, and -N(R)- in which R represents a hydrogen atom, an alkyl group or a cycloalkyl group;

30 X² represents an alkyl group having 1 to 6 carbon atoms, a carbonyl-containing group such as an acetyl group or a group of formula -(CH₂)_{n4}-CO₂H, a thiocarbonyl-containing group, a group of formula -(CH₂)_{n4}OR, a group of formula -(CH₂)_{n4}-SO₃H, or an amino-containing group such as a group of formula -(CH₂)_{n4}-(C=A)-N(R)₂ or -(CH₂)_{n4}-N(R)₂, in which A is O or S, each R is independently selected from a hydrogen atom, an alkyl group and a cycloalkyl group, and n4 is an integer of 1 to 6

35

S' is a moiety represented by the following formula:



5 wherein $sn1$ and $sn4$ are independently selected from 0 and 1, $sn2$ is selected from 2 to 8, preferably 3 to 7 and more preferably 4 to 6, and $sn3$ is selected from 1 and 2, wherein most preferably $sn1$ and $sn4$ are each 1, $sn2$ is 5, and $sn3$ is 2, and wherein $\theta 1$ indicates the position of a covalent bond to the sidechain of Lys and $\theta 2$ indicates the position of a covalent bond to Y;

10

Z is $-OH$; and

n is 1.

15 Non-cleavable linkers for use in formula (I) may for instance be selected selected from N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP); N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB) or N-succinimidyl 4-(2-pyridyldithio)-2-sulfobutanoate (sulfo-SPDB); N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC); N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfoSMCC);
 20 succinimidyl-4-(iodoacetyl)-aminobenzoate (SIAB); and N-succinimidyl-[(N-maleimidopropionamido)-tetraethyleneglycol] ester (NHS-PEG4-maleimide) and preferably SMCC or NHS-PEG4-maleimide.

3.2 Divalent group (X)

25

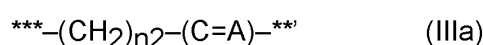
The compounds of formula (I) contain a divalent group (X) comprising one to seven, preferably two to six, more preferably two to five, e.g., 2, 3, 4 or 5, backbone atoms independently selected from C, N, O, and S; X being covalently attached to (D) via an atom selected from C, S, N and O derived from the carboxyl, thiol, amino, or hydroxyl
 30 functional group comprised in (D).

The divalent group (X) remains covalently attached to (D) after cleavage of the linker (L) by Cat B such that that a modified drug (intra drug), e.g., moiety D-X, is released into the target cell. In the present invention, it was surprisingly found that such a
 35 modified drug exhibits improved efficacy, e.g., cytotoxic efficacy, as compared with the same drug not carrying a group (X) attached thereto. Without wishing to be bound to any theory, it is believed that the spacer group (X) has a size which is appropriate, e.g., one-seven backbone atoms, for not detrimentally affecting the pharmacokinetic

properties of the conjugate even at high DAR (DAR>4), while contributing to the membrane permeation properties of the released drug moiety, e.g., due to an appropriate level of hydrophobicity. As a result, upon Cat B-induced high-rate cleavage, the drug can not only engage its molecular target(s) in the cytoplasm but
 5 also diffuse to proximal cells to induce bystander effect, e.g., cytotoxic bystander effect.

In one embodiment, especially in connection with formula (I), (X) can have the following meanings:

10 X is a group of formula (IIIa),

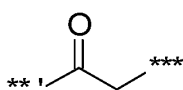


wherein n_2 is 1 or 2,

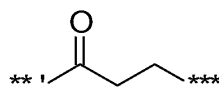
A is selected from O and S, preferably O;

*** represents covalent attachment to D; and

15 **' represents covalent attachment to the adjacent amino acid Arg, Cit or Phe; preferably a group represented by formula (IVc) or (IVd)



(IVc)

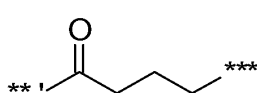


(IVd)

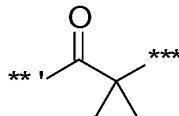
20

or

X is a group represented by formula (IVe) or (IVf)



(IVe)



(IVf)

25

*** represents covalent attachment to D;

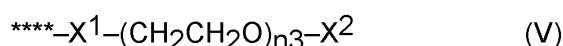
30 **' represents covalent attachment to the adjacent amino acid Arg, Cit or Phe;

30

3.3 Solubilizing moiety (S)

- The compound of formula (I) contains a solubilizing moiety (S), which is a moiety derived from a compound comprising one or more, e.g., 2, 3, 4 or 5, solubilizing groups.
- 5 The presence of a solubilizing moiety enables to reduce (or prevent) the tendency of the conjugate molecules for aggregation and thus to achieve excellent pharmacokinetic properties, e.g., biodistribution, hepatic clearance, even at high DAR (DAR>4, e.g., DAR=8). In some instances, aggregation of the conjugate molecules can be completely suppressed, even at high DAR. The present inventors have
- 10 surprisingly found that cleavage by Cat B is possible even in the presence of a sterically demanding solubilizing moiety. Preferably fast cleavage of the linker by Cat B can be accomplished, preferably by the exopeptidase mechanism of Cat B. Without being bound to any theory, it is believed that the solubilizing moiety is directed towards the outside of the Cat B binding groove, thus allowing for superior selectivity and cleavage
- 15 rate, e.g., via the exopeptidase mechanism. In addition, it was also surprisingly found that the solubilizing moiety is capable to compensate for the potential hydrophobicity of the drug moiety D-X, such that excellent pharmacokinetic properties can be retained even if multiple drug moieties are attached to the linker (e.g., $n>1$).
- 20 In one embodiment, especially in the context of formula (I), S can have the following meanings:

S is a moiety of formula (V)



- 25 wherein
 $n3$ is an integer of 6 to 200, preferably 10 to 150, more preferably 12 to 80 and in particular one or 12 to 30 or 14 to 25 or 15 to 19;

- 30 **** indicates covalent attachment to T, preferably the side chain of T;

- X^1 is selected from a single covalent bond, $-(C=O)-$, and $-N(R)-$ in which R represents a hydrogen atom, an alkyl group or a cycloalkyl group;

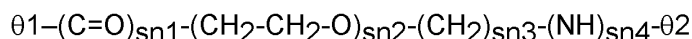
- 35 X^2 represents an alkyl group having 1 to 6 carbon atoms, a carbonyl-containing group such an acetyl group or a group of

5 formula $-(CH_2)_{n4}-CO_2H$, a thiocarbonyl-containing group, a group of formula $-(CH_2)_{n4}OR$, a group of formula $-(CH_2)_{n4}-SO_3H$, or an amino-containing group such as a group of formula $-(CH_2)_{n4}-(C=A)-N(R)_2$ or $-(CH_2)_{n4}-N(R)_2$, in which A is O or S, each R is independently selected from a hydrogen atom, an alkyl group and a cycloalkyl group, and n_4 is an integer of 1 to 6

10 In preferred aspects of this embodiment, S is represented by a moiety of formula (V), wherein X^1 is $-(C=O)-$, X^2 is a methyl group and n_3 is selected from one or the ranges of 12 to 30, 14 to 25, 14 to 20, 15 to 19, and 16 to 18.

15 In one embodiment, especially in the context of formula (I), S represents a moiety derived from a compound comprising one or more, e.g., two, three or four, solubilizing groups; wherein each solubilizing group comprised in (S) is independently selected from the group consisting of moieties comprising one or more ionic or ionizable groups, such as ammonium, guanidinium, sulfate or sulfonate groups, preferably of moieties derived from Arg, (D)-Arg, Dap, (D)-Dap, Dab, (D)-Dab, Orn, (D)-Orn, Lys, D-Lys or carnitine.

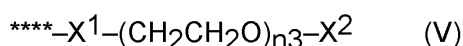
20 In the context of formula (I), S' is a moiety represented by the following formula



25 wherein n_1 and n_4 are independently selected from 0 and 1, n_2 is selected from 2 to 8, preferably 3 to 7 and more preferably 4 to 6, and n_3 is selected from 1 and 2, wherein most preferably n_1 and n_4 are each 1, n_2 is 5, and n_3 is 2, and wherein θ_1 is the position of a covalent bond to the sidechain of Lys and θ_2 is the position of a covalent bond to Y.

30 In one embodiment, (S) is a moiety derived from a compound comprising one or more polyethylene oxide groups, wherein preferably each polyethylene oxide group independently comprises from 6 to 200, more preferably from 10 to 150, most preferably from 12 to 80 repeating units.

35 In one preferred embodiment, (S) is a moiety represented by the formula (V):



wherein,

n_3 is an integer of 6 to 200, preferably 10 to 150, more preferably 12 to 80;

**** indicates covalent attachment to (T);

5

X^1 is selected from a single covalent bond, $-(C=O)-$, and $-N(R)-$ in which R represents a hydrogen atom, an alkyl group or a cycloalkyl group;

10

X^2 represents an alkyl group having 1 to 6 carbon atoms, a carbonyl-containing group such as an acetyl group or a group of formula $-(CH_2)_{n_4}-CO_2H$, a thiocarbonyl-containing group, a group of formula $-(CH_2)_{n_4}OR$, a group of formula $-(CH_2)_{n_4}-SO_3H$, or an amino-containing group such as a group of formula $-(CH_2)_{n_4}-(C=A)-N(R)_2$ or $-(CH_2)_{n_4}-N(R)_2$, in which A is O or S, each R is independently selected from a hydrogen atom, an alkyl group and a cycloalkyl group, and n_4 is an integer of 1 to 6;

15

X^2 being preferably $-CH_3$, $-CH_2CH_2OH$, or a group represented by the following formula (VI):

20



wherein,

each A is independently selected from O and S, preferably O;

25

each R is independently selected from a hydrogen atom, an alkyl group and a cycloalkyl group; and

n_5 and n_6 are each independently an integer of 1 to 6, preferably 1 or 2; and

X^2 being most preferably $-CH_3$.

30

If more than one (S) is present, each (S) is preferably a moiety of formula (V) as described above.

3.4 Branching group (T)

35

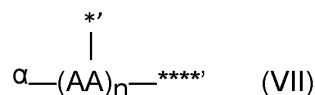
T represents a $(2+n)$ -valent, e.g., 3-, 4-, 5-, 6-valent, branching group. The branching group connects the group (V), the solubilizing moiety (S) and one or more (n) linker moieties (L) thereby forming a branched structure. Preferably, T is a 3-valent ($n=1$), or 4-valent ($n=2$) branching group. More preferably, T is a 3-valent branching group.

In one embodiment, especially in the context of formula (I), T has the following meaning:

5 T is derived from an amino acid selected from N- ϵ -propargyloxycarbonyl-L-Lysine (Lys(Poc)), Asp, Glu, Orn, Lys, Dab and Dap, preferably derived from Lys(Poc), Glu, Orn or Lys, more preferably derived from Lys.

In one embodiment, the branching group is a group comprising at least one moiety derived from a trifunctional amino acid, e.g., from Lys. The branching group can
10 comprise further (optional) linkers and/or amino acids in addition to the trifunctional amino acid mentioned above, provided that the said further linkers do not contain a solubilizing group, such as a polyalkylene oxide group, and/or that the said further amino acids are not trifunctional amino acids or moieties comprising one or more ionic or ionizable groups. Such further amino acids can, for example, be selected from
15 homo-Phe (hPhe) and Phe. In some aspects, the branching group consists of a moiety derived from a trifunctional amino acid (i.e., does not include any further linkers and/or amino acids).

In one embodiment, (T) is a moiety represented by the following formula (VII):
20



wherein,
25 each AA independently represents a moiety derived from a trifunctional amino acid such as a diamino-carboxylic acid, an amino dicarboxylic acid, an azido amino acid or an alkyne-containing amino acid, preferably derived from an amino acid selected from N- ϵ -propargyloxycarbonyl-L-Lysine (Lys(Poc)), Asp, Glu, Orn, Lys, Dab and Dap, more preferably derived from Lys(Poc), Glu, Orn or Lys, most
30 preferably derived from Lys;

α indicates covalent attachment to (Y);

if $n = 1$, the side chain originating from the trifunctional amino acid is covalently attached to (L) or (S), the C-terminus is covalently attached to the other moiety (S) or (L), respectively;

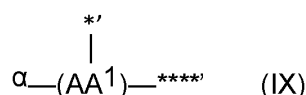
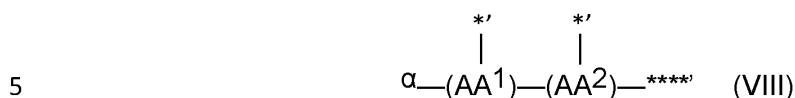
35 if $n = 2, 3$ or 4 :

*' indicates covalent attachment to (L);

****' indicates covalent attachment to (S); and

n is as defined in formula (I).

In one further embodiment, (T) is a moiety represented by the formula (VIII) or (IX):



10 wherein,
each AA¹ and AA² is independently a moiety derived from a trifunctional amino acid, such as a diamino-carboxylic acid, an amino dicarboxylic acid, an azido amino acid or an alkyne-containing amino acid, preferably a moiety derived from an amino acid selected from Lys(Poc), Asp, Glu, Orn, Lys, Dab and Dap, more
15 preferably a moiety derived from Lys(Poc), Glu, Orn or Lys, most preferably a moiety derived from Lys;
 α indicates covalent attachment to (Y);
in formula (IX), the side chain originating from the trifunctional amino acid is covalently attached to (L) or (S), the C-terminus is covalently attached to the other moiety (S) or (L), respectively;
20 in formula (VIII), *' indicates covalent attachment to (L), and *****' indicates covalent attachment to (S).

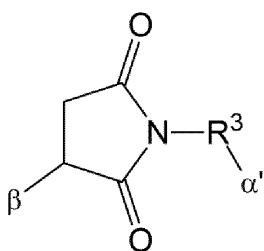
3.5 Divalent group (Y)

25 The compound of formula (I) contains a divalent group (Y) comprising one or more atoms selected from C, N, O, P and S. The divalent group connects the group (V) to the branching group (T). The divalent group is typically attached to the side chain of an amino acid contained in group (V), such as Cys. Preferably, Y is a divalent group
30 derived from a compound selected from maleimides, triazoles, hydrazones, carbonyl-containing compounds, and derivatives thereof. More preferably, Y is a divalent group derived from maleimides and derivatives thereof, such as opened hydrolyzed maleimide derivatives. Most preferably, Y is a divalent group derived from an opened hydrolyzed maleimide.

35 In some embodiments, especially in the context of formula (I), Y has the following meaning:

Y is a group derived from a compound selected from maleimides, triazoles, hydrazones, carbonyl-containing compounds and derivatives thereof, preferably from maleimides and derivatives thereof such as opened hydrolyzed maleimide derivatives, and more preferably derived from an opened hydrolyzed maleimide.

Preferably, Y in the conjugate compound of formula (I) is represented by a divalent group represented by any of the following formula (XIIIa):



Formula (XIIIa)

wherein,

R^3 represents $-(CH_2)_{n7}-(C=A)_{n9}-\alpha'$ or $-(CH_2)_{n7}-(C=A)_{n9}-NH-(CH_2CH_2O)_{n8}-(C=A)_{n9}-\alpha'$, preferably $-(CH_2)_{n7}-(C=A)_{n9}-\alpha'$, wherein,
 $n7$ is 1 or 2, preferably 1,
 $n8$ is 1 to 6, preferably 1,
 $n9$ is 0 or 1, preferably 1, and
 A is O or S, preferably O;

wherein the methylene carbon atom of R^3 is covalently attached to the nitrogen atom of formulae (XIIIa) and the carbonyl or thiocarbonyl-carbon is covalently attached to T;

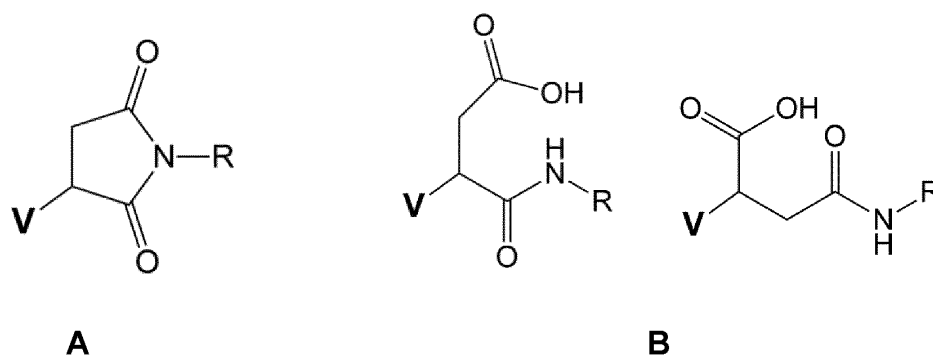
β indicates covalent attachment to V; and

α' indicates covalent attachment to T, preferably by amide bond formation with the amino group that is attached to $C\alpha$ of the amino acid.

Hydrolysis of a maleimide attachment to V is typically performed under basic conditions as a final step of the conjugation of the maleimide derivative to V, as in general procedure 1 to 3 set out in the examples herein. The following conditions are especially suitable: at the end of a cysteine maleimide conjugation reaction, pH is adjusted to pH 8 by adding 10x pH 8 DPPS (0.2 to 0.5 reaction volume) and excess reactive drug linker and reducing agent (TCEP) are removed via gel filtration using suitable columns for gel filtration (PF column, elution with pH 8 buffer). The eluent is then stirred

overnight for 16h to complete the opening before final buffer exchange with DPBS into an Amicon concentrating unit.

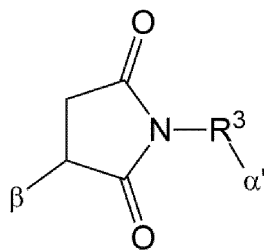
In some instances, when m in the compound is at least 2, said compound may comprise a mix of (closed) maleimide derivatives (Y) and opened hydrolyzed maleimide derivatives (Y) attached to V. Accordingly, in the compounds described herein in which a group (R) is attached to a vector (V) via a maleimide (as shown below, left hand side), hydrolysis may be carried out such that, when m is at least 2, a compound of the invention may comprise both closed maleimide attachments (A) and opened hydrolyzed maleimide attachments (B) (as shown below, left hand side) to V.



In preferred embodiments, wherein m is at least 2, at least 50% of the Y attachments to V are opened hydrolyzed maleimide attachments (B), the remaining attachments being closed maleimide attachments (A). In some instances, at least 70%, at least 75%, at least 80%, at least 90%, at least 95%, preferably at least 98% of the Y attachments to V are opened hydrolyzed maleimide attachments (B).

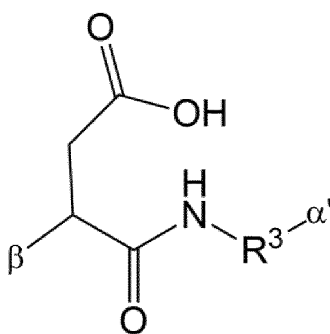
The presence of one or more opened hydrolyzed maleimide attachments (even if present together with one or more closed maleimide attachments) can contribute to the stability and therapeutic efficacy of the compounds of the invention. Without being bound to any theory, it is believed that the opened hydrolyzed maleimide attachment e.g., prevents a retro-Michael reaction (as shown in Fig. 29) that causes the liberation of reactive maleimide in the circulation and ultimately leads to transfer of linker-payload to other thiol-containing molecule in the body, such as albumin. The opened maleimide may also cooperate with the divalent group X and the solubilizing moiety S to achieve improved stability and therapeutic efficacy.

In a preferred embodiment, Y is a divalent group derived from maleimides and derivatives thereof, such as opened hydrolyzed maleimides, preferably a divalent group represented by any of the following formulae (XIIIa) to (XIIIc):

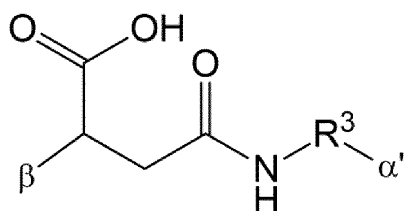


Formula (XIIIa)

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Formula (XIIIb)



Formula (XIIIc)

- 10 wherein,
 R^3 represents $-(CH_2)_{n7}-(C=A)_{n9}-$ or $-(CH_2CH_2O)_{n8}-(C=A)_{n9}-$, preferably $-(CH_2)_{n7}-(C=A)_{n9}-$, wherein,
 15 $n7$ is 1 to 6, preferably 1 or 2, more preferably 1,
 $n8$ is 1 to 6, preferably 1,
 $n9$ is 0 or 1, preferably 1, and
 A is O or S, preferably O;

wherein the methylene carbon atom is covalently attached to the nitrogen atom of formulae (XIIIa)-(XIIIc) and the carbonyl or thiocarbonyl-carbon is covalently attached to T;

β indicates covalent attachment to V; and

5 α' indicates covalent attachment to T.

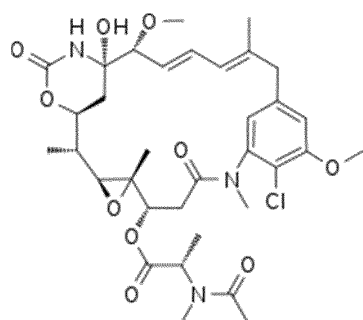
In a more preferred embodiment, Y is represented by formula (XIIIb) or (XIIIc), wherein R^3 is preferably a group represented by the formula $-(CH_2)_{n7}-(C=A)_{n9}-$ in which $n7$ is 1 or 2, $n9$ is 1 and A is O. Most preferably, R^3 is $-CH_2-C=O-$.

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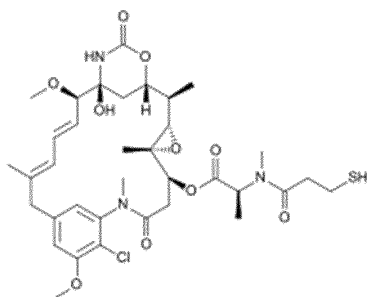
3.6 Moiety (D)

The compound of formula (I) contains a moiety derived from a drug, wherein the drug is selected from maitansine, mertansine (DM1) and raptansine (DM4), preferably DM1 or DM4 and more preferably DM1. The structures of maitansine, DM1 and DM4, respectively, are shown below:

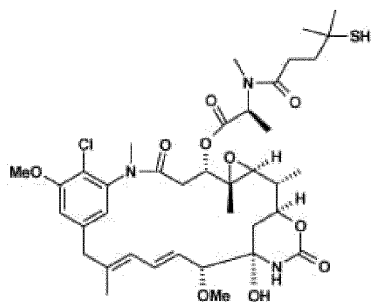
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Maitansine



DM1 (mertansine)



DM4 (ravtansine)

If more than one (D) is present in the compound of formula (I) ($n > 1$ and/or $m > 1$), each (D) is independently selected. Nonetheless, it is preferred that the multiple moieties
 5 (D) are identical to each other.

The drug can be unmodified (in its natural form except for the replacement of a hydrogen atom by a covalent bond), or can be chemically modified in order to incorporate one or more functional groups (e.g. one or more groups selected from
 10 hydroxyl, carboxyl, amino and thiol groups) allowing covalent attachment(s) to the divalent group (X), the drug moiety, e.g., moiety D-X or moiety D-X-Dxx-Dyy, being preferably pharmacologically active once it is released from the conjugate. According to one embodiment, the drug moiety that is released from the conjugate, e.g., moiety
 15 D-X or moiety D-X-Dxx-Dyy, is pharmacologically active in such a sense that it retains at least 20%, preferably at least 35%, more preferably at least 50%, and even more preferably at least 70% of the pharmacological activity of the corresponding unmodified (native) drug.

3.7 Group (V)

20 The group (V) in formulae (I), (X) and (X') represents a moiety derived from a vector group capable of interacting with a target cell by targeting CD37. The expression "capable of interacting with a target cell by targeting CD37" as used herein indicates that the group can bind to, complex with, or react with leukocyte antigen CD37 on the
 25 surface of a target cell. Such an interaction with the target cell can be experimentally verified by methods known in the art, for instance by providing a compound of formula (I), which carries a label (such as a fluorescence marker), by contacting said compound with tissue containing target cells and by detecting the distribution of the fluorescence marker within the tissue (e.g., by fluorescence microscopy). An increase of
 30 fluorescence intensity at the target cells indicates an interaction with the target cell in accordance with the present invention. In some preferred embodiments, the vector

group is also capable of causing or contributing to internalization of the targeted-drug-conjugate, i.e., the compound of formula (I), into the target cell.

As used herein, the term "anti-CD37 antibody" (or "an antibody that binds to CD37" or the like) refers to an antibody capable of binding CD37 with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting the antigen CD37. The extent of binding of an anti-CD37 antibody to a respective unrelated, non-CD37 protein can be less than about 10% of the binding of the antibody to CD37 as measured, e.g., by a radioimmunoassay (RIA). In certain embodiments, an antibody that binds to any of CD37 has a dissociation constant (K_d) of $\leq 1 \mu\text{M}$, $\leq 1100 \text{ nM}$, $\leq 110 \text{ nM}$, $\leq 11 \text{ nM}$, or $\leq 1.1 \text{ nM}$.

In specific embodiments, (V) represents a moiety derived from a group selected from the naratuximab antibody, as well as antibody fragments, and Fc fusion proteins derived from naratuximab.

In one embodiment, (V) represents a moiety derived from the naratuximab antibody or a naratuximab antibody fragment such as a naratuximab-derived single chain antibody, monoclonal antibody, single chain monoclonal antibody, monoclonal antibody fragment, chimeric antibody, chimeric antibody fragment or domain antibody or fragment thereof.

In other embodiments, (V) represents an antibody fragment incorporated into an Fc-fusion protein.

As used herein, the term "naratuximab" (also referred to as K7153A herein) refers to the antibody huCD37-3 (Version 1.0) described in WO 2019/229677 (incorporated herein by reference). In another aspect, naratuximab is characterized as the monoclonal antibody huCD37-3v1.0 in WO2011/112978, which specifically describes its heavy chain (SEQ ID NO:90) and light chain (SEQ ID 107). In yet further aspects, said antibody comprises the CDRs represented by SEQ ID NOs:2-7 in tables 1 and 2, the VH of SEQ ID NO:8 in table 3 and the VL of SEQ ID NO:10 in table 4 of WO 2019/229677.

In particular aspects, naratuximab may comprise

- the full-length light chain of Seq No 11 in table 5 of WO 2019/229677,
- the full-length heavy chain of Seq No. 12 in table 6 of WO 2019/229677,

- the full-length light chain of Seq No 11 in table 5 and the full-length heavy chain of Seq No. 12 in table 6 of WO 2019/229677,
- a light chain or light chain variable region having the same amino acid sequence as the amino acid sequence encoded by the recombinant plasmid DNA phuCD37-3LC (ATCC Deposit Designation PTA-10722, deposited with the ATCC on March 18, 2010),
- a heavy chain or heavy chain variable region comprising the same amino acid sequence as the amino acid sequence encoded by the recombinant plasmid DNA phuCD37-3HCv.1.0 (ATCC Deposit Designation PTA-10723, deposited with the ATCC on March 18, 2010),
- a light chain or light chain variable region comprising the same amino acid sequence as the amino acid sequence encoded by the recombinant plasmid DNA phuCD37-3LC (PTA-10722) and a heavy chain or heavy chain variable region comprising the same amino acid sequence as the amino acid sequence encoded by the recombinant plasmid DNA phuCD37-3HCv.1.0 (PTA-10723),
- comprise (i) VL-CDRs comprising the same amino acid sequences as the VL-CDRs encoded by the recombinant plasmid DNA phuCD37-3LC (PTA-10722) and (ii) VH-CDRs comprising the same amino acid sequences as the VH-CDRs encoded by the recombinant plasmid DNA phuCD37-3HCv.1.0 (PTA-10723).

In one embodiment, (V) represents a moiety derived from the anti-CD37 antibody naratuximab.

Other groups targeting CD37 may also be used as or in the (V) group of the conjugate compound. This includes for instance the anti-CD37 antibody AGS67C. The preparation of this antibody is described in the experimental section of Pereira DS, et al. AGS67E, an Anti-CD37 Monomethyl Auristatin E Antibody-Drug Conjugate as a Potential Therapeutic for B/T-Cell Malignancies and AML: A New Role for CD37 in AML. *Mol Cancer Ther.* 2015 Jul;14(7):1650-60. doi: 10.1158/1535-7163.MCT-15-0067. Another suitable anti-CD37 agent is otlertuzumab (TRU-016), which is commercially available and described in Pagel JM, et al. Otlertuzumab (TRU-016), an anti-CD37 monospecific ADAPTIR[™] therapeutic protein, for relapsed or refractory NHL patients. *Br J Haematol.* 2015 Jan;168(1):38-45. doi: 10.1111/bjh.13099. Further CD-37 targeting molecules are described in the patent literature, such as CA2693464A1, CA2799036A1, AU201406229A1, EP2790740A1, EP3601354A1,

WO2014143807A2, WO2020216947A1, WO2021087248A1, WO2022235589A1, WO2023068226A1, or US2016051694A1.

3.8 Conjugate compounds of formula (I)

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The conjugate compound of formula (I) is represented by the following formula:



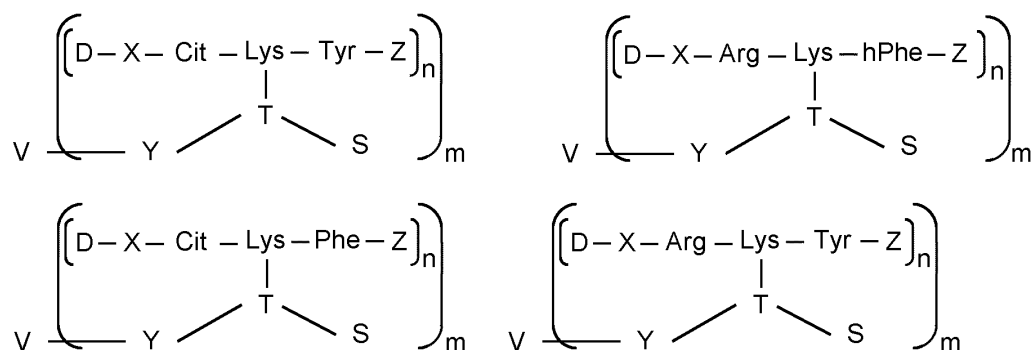
10 wherein V represents an anti-CD37 antibody, a fragment or derivative thereof or Fc-fusion protein containing an anti-CD37 antibody fragment and preferably naratuximab, a fragment or derivative thereof or Fc-fusion protein containing a naratuximab fragment,

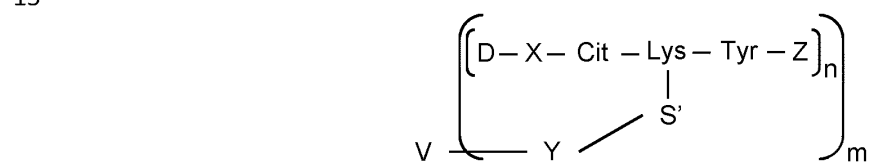
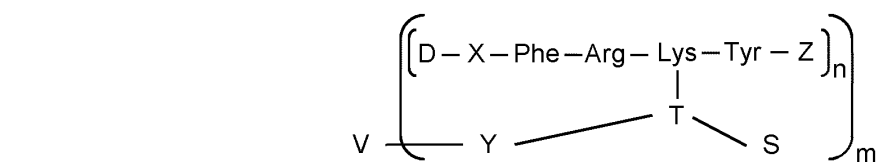
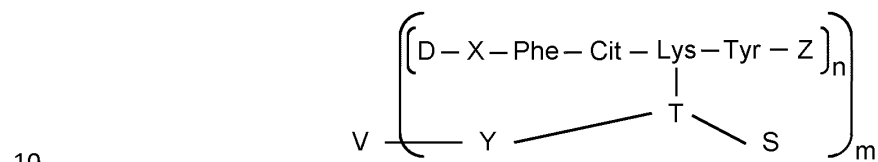
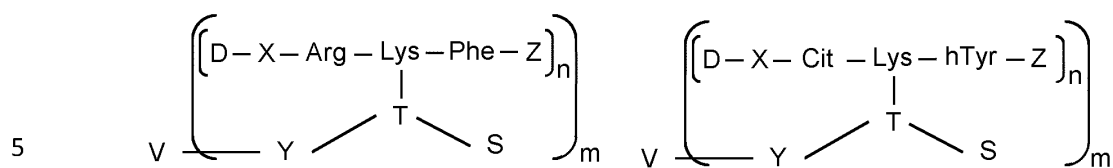
15 LL represents a linker covalently bonded to V and D,

D represents a moiety derived from a drug selected from maytansine, mertansine (DM1) and raptansine (DM4), preferably DM1 or DM4 and more preferably DM1; and

20 m is an integer of 1 to 12, preferably 2 to 10 and more preferably 4 to 8.

In preferred embodiments, the conjugate compound of formula (I) is a compound represented by one of the following formulae:

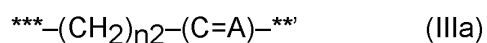




wherein

D has the same meaning as specified in claim 1, and preferably D is a moiety derived from DM1 or DM4;

X is a group of formula (IIIa),

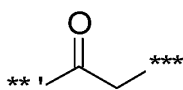


wherein n_2 is 1 or 2,

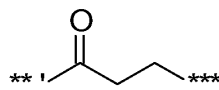
A is selected from O and S, preferably O;

*** represents covalent attachment to D; and

**' represents covalent attachment to the adjacent amino acid Arg, Cit or Phe; preferably a group represented by formula (IVc) or (IVd)



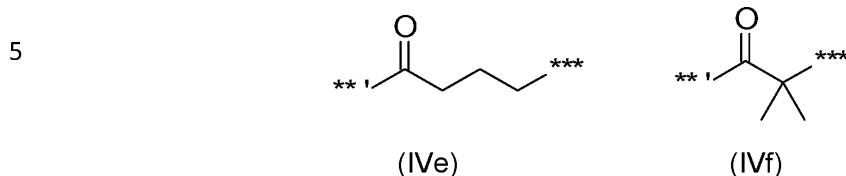
(IVc)



(IVd)

or

X is a group represented by formula (IVe) or (IVf)



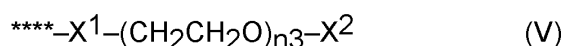
*** represents covalent attachment to D;

10 ** represents covalent attachment to the adjacent amino acid Arg, Cit or Phe;

15 Y is a group derived from a compound selected from maleimides, triazoles, hydrazones, carbonyl-containing compounds and derivatives thereof, preferably from maleimides and derivatives thereof such as opened hydrolyzed maleimide derivatives, and more preferably derived from an opened hydrolyzed maleimide;

20 T is derived from an amino acid selected from N-ε-propargyloxycarbonyl-L-Lysine (Lys(Poc)), Asp, Glu, Orn, Lys, Dab and Dap, preferably derived from Lys(Poc), Glu, Orn or Lys, more preferably derived from Lys;

S is a moiety of formula (V)



wherein

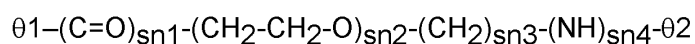
25 n3 is an integer of 6 to 200, preferably 10 to 150, more preferably 12 to 80 and in particular one or 12 to 30 or 14 to 25 or 15 to 19;

30 **** indicates covalent attachment to T, preferably the side chain of T;

X¹ is selected from a single covalent bond, -(C=O)-, and -N(R)- in which R represents a hydrogen atom, an alkyl group or a cycloalkyl group;

X^2 represents an alkyl group having 1 to 6 carbon atoms, a carbonyl-containing group such as an acetyl group or a group of formula $-(CH_2)_{n4}-CO_2H$, a thiocarbonyl-containing group, a group of formula $-(CH_2)_{n4}OR$, a group of formula $-(CH_2)_{n4}-SO_3H$, or an amino-containing group such as a group of formula $-(CH_2)_{n4}-(C=A)-N(R)_2$ or $-(CH_2)_{n4}-N(R)_2$, in which A is O or S, each R is independently selected from a hydrogen atom, an alkyl group and a cycloalkyl group, and n_4 is an integer of 1 to 6

S' is a moiety represented by the following formula

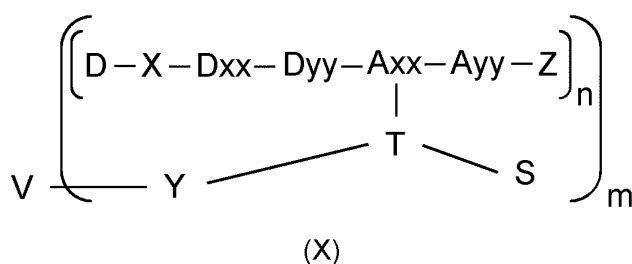


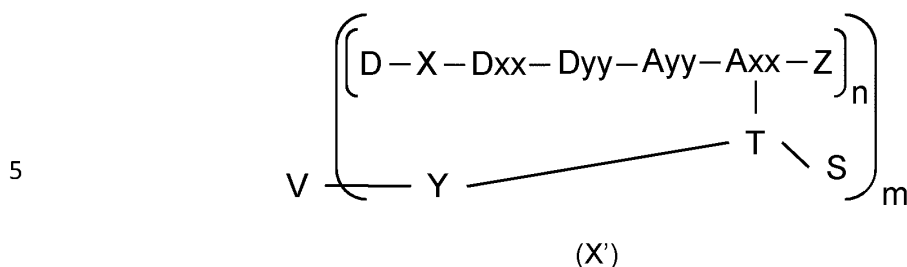
wherein sn_1 and sn_4 are independently selected from 0 and 1, sn_2 is selected from 2 to 8, preferably 3 to 7 and more preferably 4 to 6, and sn_3 is selected from 1 and 2, wherein most preferably sn_1 and sn_4 are each 1, sn_2 is 5, and sn_3 is 2, and wherein θ_1 is the position of a covalent bond to the sidechain of Lys and θ_2 is the position of a covalent bond to Y;

Z is $-OH$; and

n is 1.

The conjugate compound of formula (I) may, in some instances, be represented by the following general formula (X) or (X'):





10 Axx in formula (X) and in formula (X') represents a moiety derived from an amino acid selected from Glu, Apa, Aaa, Dap, Dab, Lys, Orn, Ser, Ama and homo-Lys, preferably a moiety derived from an amino acid selected from Dap, Dab, Lys, Orn and homo-Lys, more preferably a moiety derived from a moiety derived from Lys;

15 Ayy in formula (X) represents a moiety derived from an amino acid selected from Phe, homo-Phe, Ala, Trp, Phg, Leu, Val, Tyr, homo-Tyr, Tyr(OR₁) and homo-Tyr(OR₁) wherein R₁ is $-(CH_2CH_2O)_{n1}-R_2$, wherein R₂ is a hydrogen atom or a methyl group and n1 is an integer of 2 to 24, preferably a moiety derived from Phe, homo-Phe, Tyr, homo-Tyr, Tyr(OR₁) or homo-Tyr(OR₁), more preferably a moiety derived from Tyr;

20 Ayy in formula (X') represents a moiety derived from an amino acid selected from Phe, homo-Phe, Ala, Trp, Phg, Leu, Val, Tyr and Ser, preferably a moiety derived from Phe, home-Phe or Ser, more preferably a moiety derived from Phe or Ser;

25 D, Dxx, Dyy, X, T, S, V, Z, m and n in formulae (X) and (X') have the same meanings as described herein.

According to one preferred embodiment, at least one, e.g., two, three, four, five, six, seven or eight, of D, Dxx, Dyy, X, Y, T, S and Z is/are defined as follows:

- 30 (a) D is a moiety derived from a drug selected from maytansine, DM1 and DM4, preferably a moiety derived from DM1 or DM4;
- (b) Dxx is a moiety derived from an amino acid selected from Phe, Val, Tyr, homo-Phe and Ala, preferably from Phe or Val;
- 35 (c) Dyy is a covalent bond or a moiety derived from an amino acid selected from Arg, Lys, Cit, Orn, Dap and Dab, preferably a covalent bond or a moiety derived from Arg or Cit;

- (d) X is a group of formula (IIIa) wherein n₂ is 1 or 2, or a group represented by any of formulae (IVc) to (IVf);
- (e) Y is a group derived from a compound selected from maleimides, triazoles, hydrazones, carbonyl-containing compounds and derivatives thereof, preferably a group derived from maleimides and derivatives thereof such as opened hydrolyzed maleimide derivatives, and more preferably from an opened hydrolyzed maleimide;
- (f) T is a group of formula (VII), (VIII) or (IX);
- (g) S is a moiety of formula (V); and
- (h) Z is –OH.

According to one preferred embodiment, at least one, e.g., two, three, four, five, six, seven, eight or nine, of D, Dxx, Dyy, X, Y, T, S, V and Z is/are defined as follows:

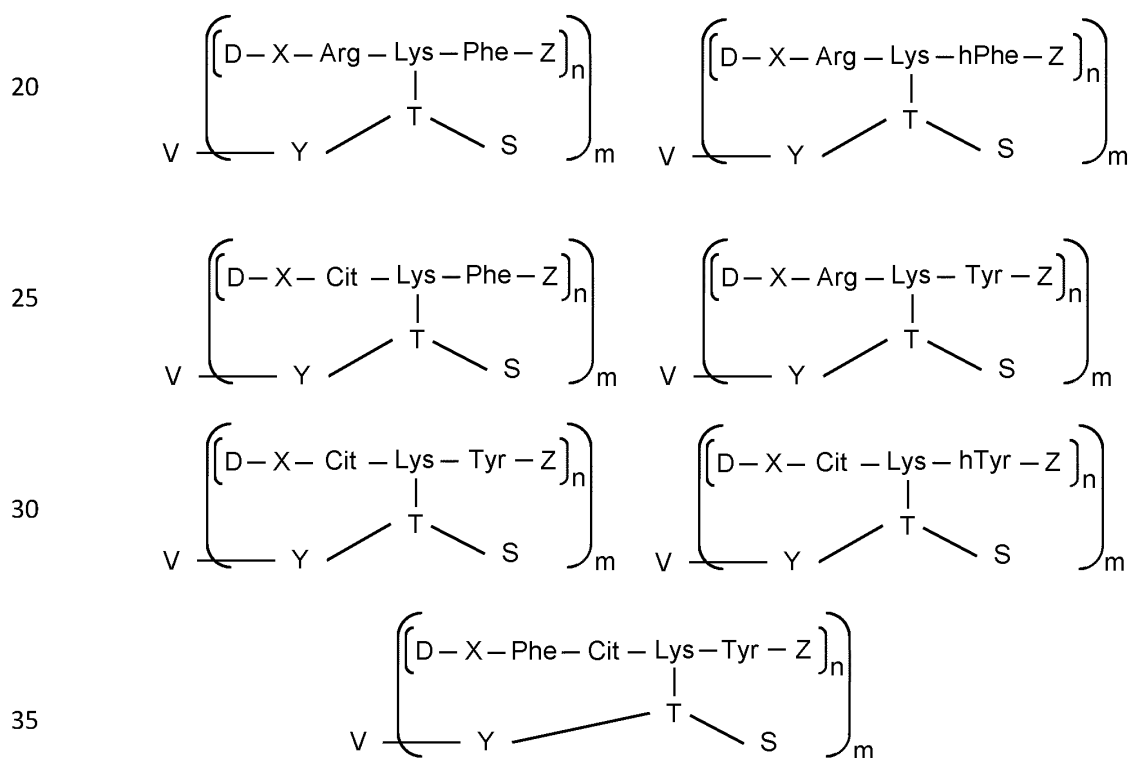
- (a) D is a moiety derived from a drug selected from maytansine, DM1 and DM4, preferably a moiety derived from DM1 or DM4;
- (b) Dxx is a moiety derived from an amino acid selected from Phe, Val, Tyr, homo-Phe and Ala, preferably from Phe or Val;
- (c) Dyy is a covalent bond or a moiety derived from an amino acid selected from Arg, Lys, Cit, Orn, Dap and Dab, preferably a covalent bond or a moiety derived from Arg or Cit;
- (d) X is a group of formula (IIIa) wherein n₂ is 1 or 2, or a group represented by any of formulae (IVc) to (IVf);
- (e) Y is a group derived from a compound selected from maleimides, triazoles, hydrazones, carbonyl-containing compounds and derivatives thereof, preferably a group derived from maleimides and derivatives thereof such as opened hydrolyzed maleimide derivatives, and more preferably from an opened hydrolyzed maleimide;
- (f) T is a group of formula (VII), (VIII) or (IX);
- (g) S is a moiety of formula (V);
- (h) V is a moiety derived from naratuximab; and

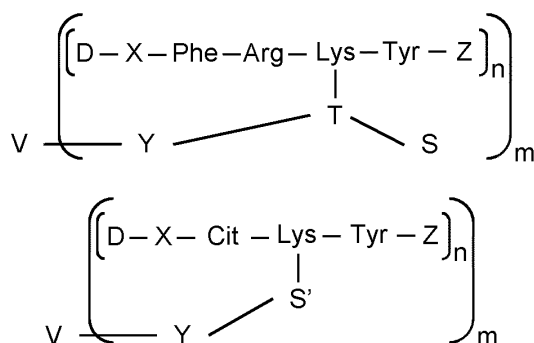
(i) Z is -OH.

According to a further preferred embodiment, each Dyy-Dxx-Axx-Ayy in formula (X) is independently selected from Arg-Lys-Phe wherein Dyy is a covalent bond, Arg-Lys-homoPhe wherein Dyy is a covalent bond, Arg-Lys-Tyr wherein Dyy is a covalent bond, Cit-Lys-Phe wherein Dyy is a covalent bond, Cit-Lys-Tyr wherein Dyy is a covalent bond, Arg-Lys-homoTyr wherein Dyy is a covalent bond, Cit-Lys-homoTyr wherein Dyy is a covalent bond, Phe-Cit-Lys-Phe, Phe-Cit-Lys-Tyr, Phe-Arg-Lys-Tyr, Phe-Cit-Lys-homoTyr, Phe-Lys-Lys-Phe, homoPhe-Arg-Lys-Phe, homo-Phe-Cit-Lys-Tyr; and each Dyy-Dxx-Ayy-Axx in formula (X') is independently selected from Arg-Phe-Lys wherein Dyy is a covalent bond, Arg-Ser-Lys wherein Dyy is a covalent bond, Cit-Phe-Lys wherein Dyy is a covalent bond, Cit-Ser-Lys wherein Dyy is a covalent bond, Cit-homoPhe-Lys wherein Dyy is a covalent bond, Phe-Cit-Phe-Lys, homoPhe-Cit-Phe-Lys, and Phe-Arg-Phe-Lys.

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In one embodiment, the compound of the present invention is represented by one of the following formulae:





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wherein D, X, Y, T, S, S', V, Z, m and n have the same meanings as described above.

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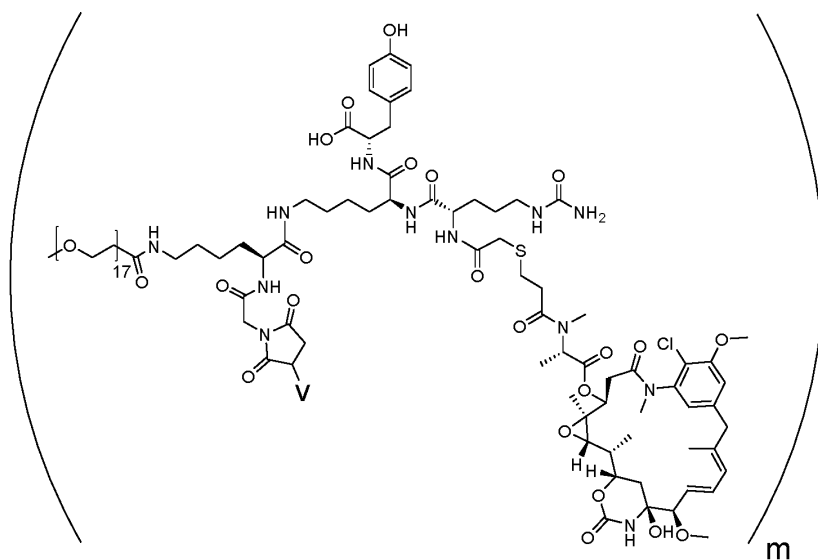
In preferred embodiments, the compound of the present invention is represented by one of the following formulae (as is evident from formula (I), in the following formulae, the group (V) is to be understood as being outside the parentheses so that indicia m does not apply to (V) whereas all other depicted structural elements are to be understood as being within the parentheses and thus present in the molecule m times):

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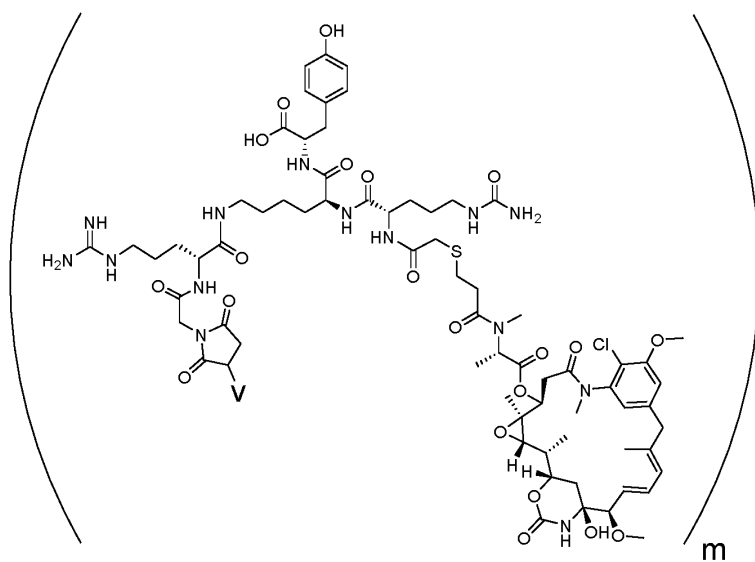
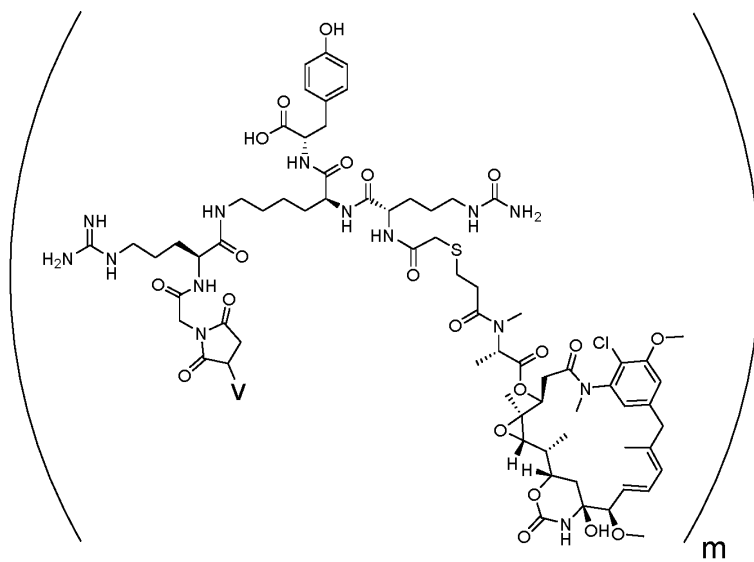
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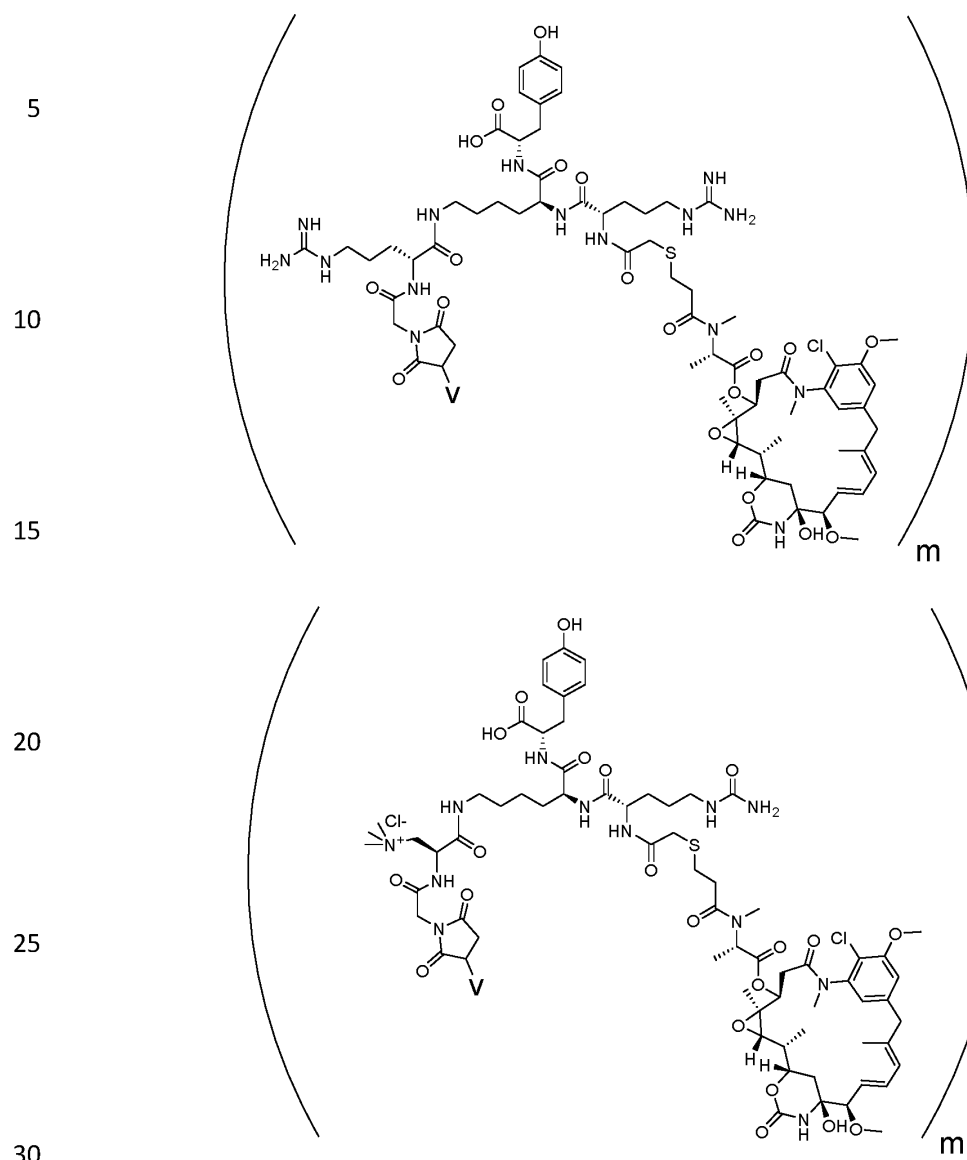


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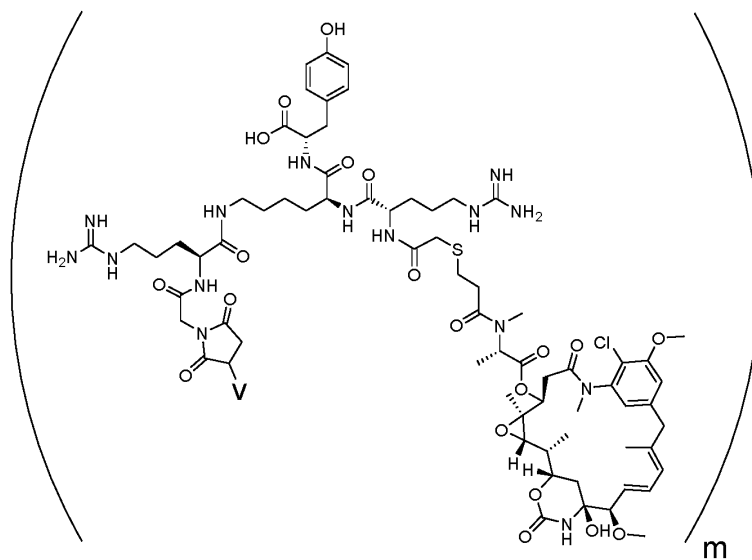




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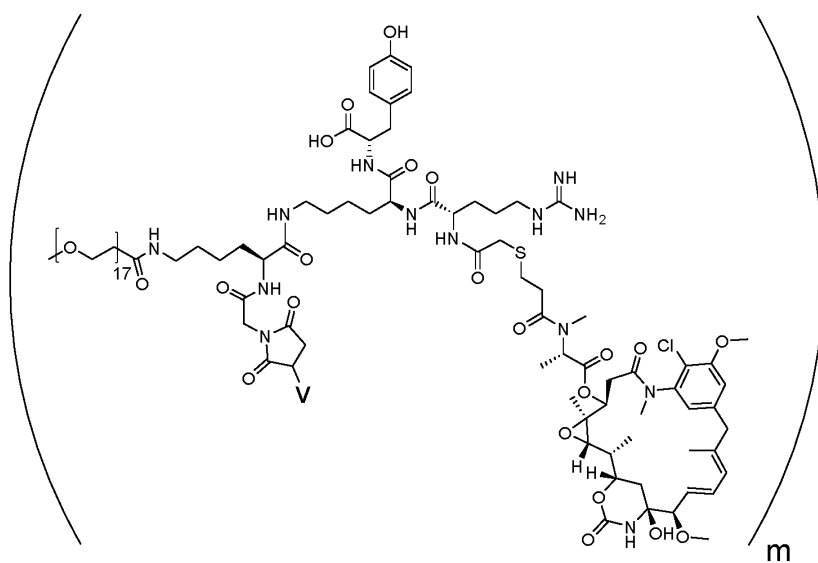
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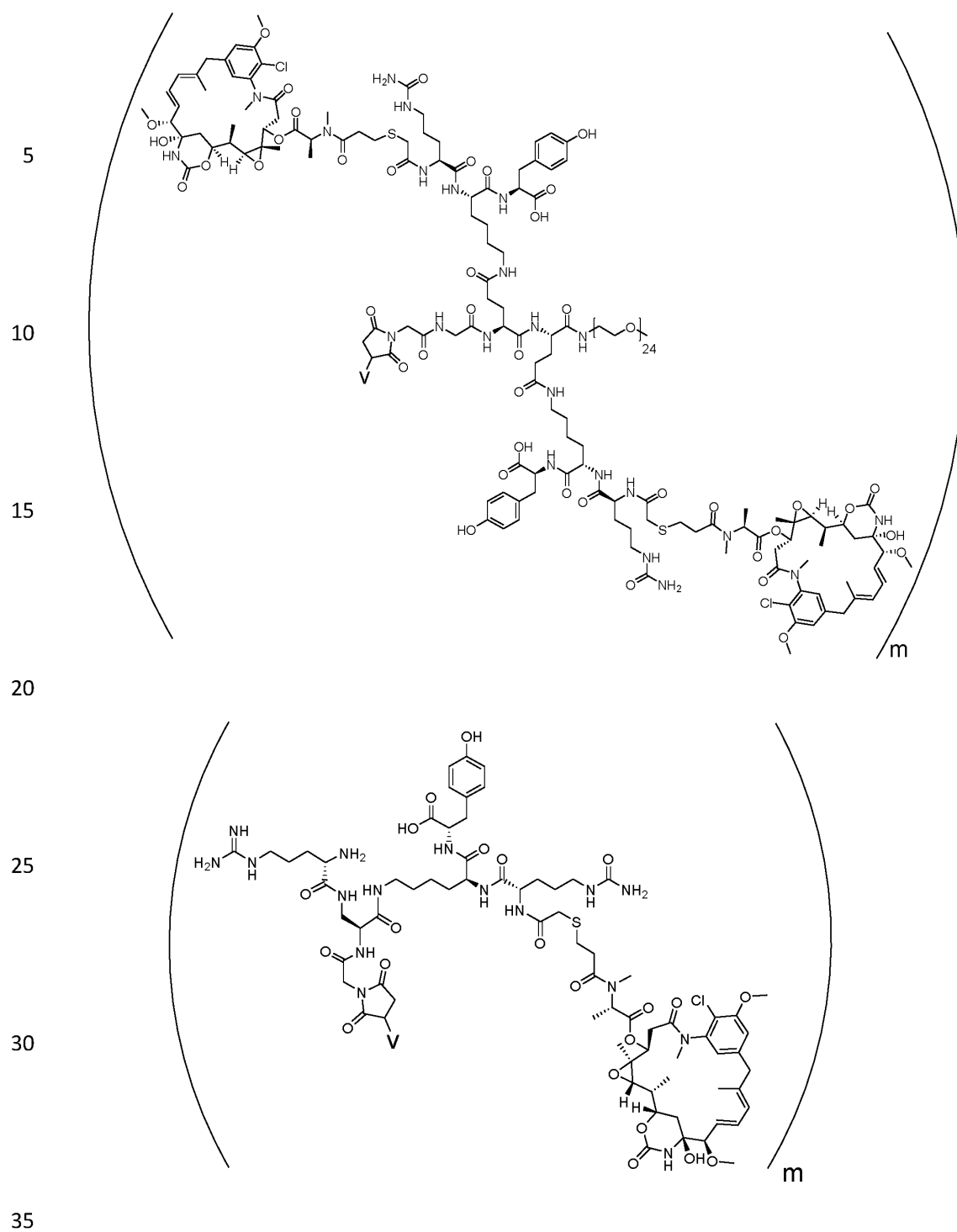
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wherein m is an integer of 1 to 12, preferably 2 to 10, more preferably 4 to 8.

According to one embodiment, in the above compounds wherein the solubilizing moiety (S) comprises a C₂ polyoxyalkylene oxide group, the number of oxyethylene repeating units (17) may be replaced by 12 to 22, preferably 15 to 19 oxyethylene groups.

5

According to one embodiment, in the above compounds the maleimide attachment to (V) may be replaced by an opened hydrolyzed maleimide attachment. In some instances, wherein m is at least 2, said compound may comprise a mix of (closed) maleimide derivatives and opened hydrolyzed maleimide derivatives attached to V, preferably at least 50% of the attachments to V are opened hydrolyzed maleimide attachments.

10

In one preferred embodiment, the conjugate compound (LDC) is represented by one of the following formulae:

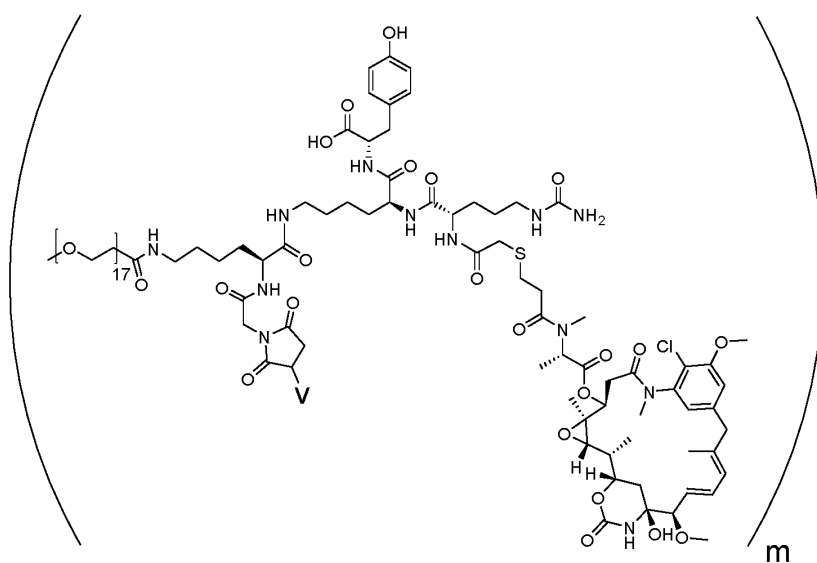
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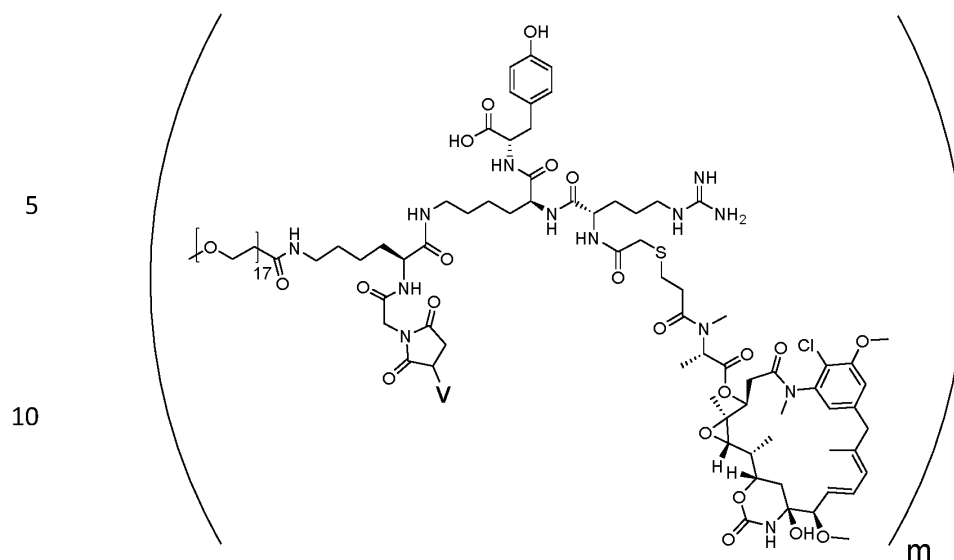
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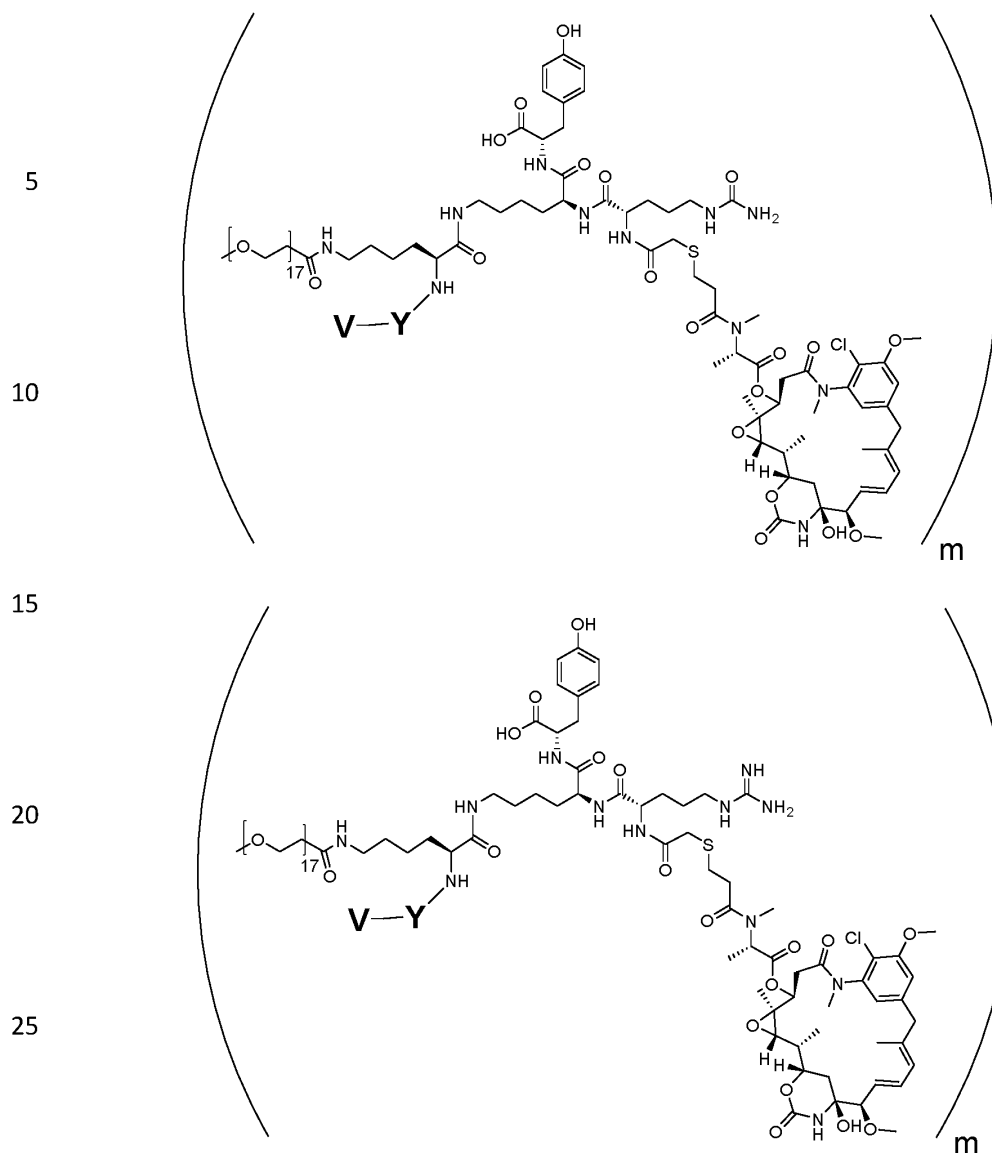
wherein m is an integer of 1 to 12, preferably 2 to 10, more preferably 4 to 8.

As is evident from formula (I), in the above compounds, the group (V) is to be understood as being outside the parentheses so that indicia m does not apply to (V) whereas all other depicted structural elements are to be understood as being within the parentheses and thus present in the molecule m times.

According to one embodiment, in the above compounds wherein the solubilizing moiety (S) comprises a C_2 polyoxyalkylene oxide group, the number of oxyethylene repeating units (17 or 24) may be replaced by 12 to 30, preferably 14 to 25, more preferably 15 to 19 oxyethylene groups.

According to one embodiment, in the above compounds the maleimide attachment to (V) may be replaced by an opened hydrolyzed maleimide attachment. In some instances, wherein m is at least 2, said compound may comprise a mix of (closed) maleimide derivatives and opened hydrolyzed maleimide derivatives attached to V, preferably at least 50% of the attachments to V are opened hydrolyzed maleimide attachments.

In one preferred embodiment, the conjugate compound (LDC) is represented by one of the following formulae:



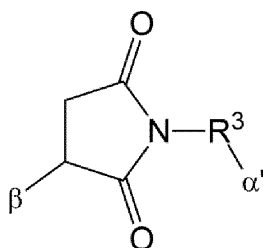
30 wherein,
V is as defined in formula (I);
Y is a group derived from maleimides and derivatives thereof such as opened
hydrolyzed maleimide derivatives, preferably from an opened hydrolyzed
maleimide; and
35 m is an integer of 1 to 12, preferably 2 to 10, more preferably 4 to 8.

As is evident from formula (I), in the above compounds, the group (V) is to be understood as being outside the parentheses so that indicia m does not apply to (V)

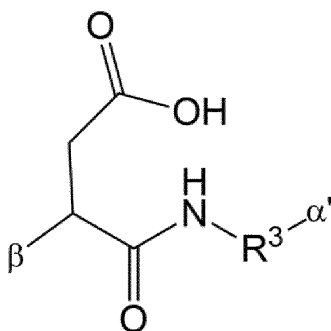
whereas all other depicted structural elements are to be understood as being within the parentheses and thus present in the molecule m times.

According to one embodiment, in the above compounds wherein the solubilizing moiety (S) comprises a C_2 polyoxyalkylene oxide group, the number of oxyethylene repeating units (17 or 24) may be replaced by 12 to 30, preferably 14 to 25, more preferably 15 to 19 oxyethylene groups.

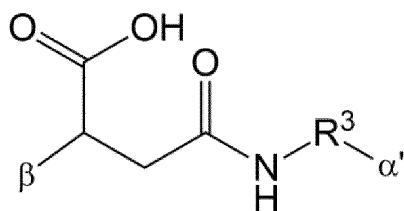
According to a preferred embodiment, in the above compounds, Y is represented by any of the following formulae (XIIIa) to (XIIIc):



Formula (XIIIa)



Formula (XIIIb)



Formula (XIIIc)

wherein,

R^3 represents $-(CH_2)_{n7}-(C=A)_{n9}-$ or $-(CH_2CH_2O)_{n8}-(C=A)_{n9}-$, preferably $-(CH_2)_{n7}-(C=A)_{n9}-$, wherein,

$n7$ is 1 to 6, preferably 1 or 2, more preferably 1,

$n8$ is 1 to 6, preferably 1,

$n9$ is 0 or 1, preferably 1, and

A is O or S, preferably O;

wherein the methylene carbon atom is covalently attached to the nitrogen atom of formulae (XIIIa)-(XIIIc) and the carbonyl or thiocarbonyl-carbon is covalently attached to the nitrogen atom of T (to the amino group derived from the Lys residue of T);

β indicates covalent attachment to V; and

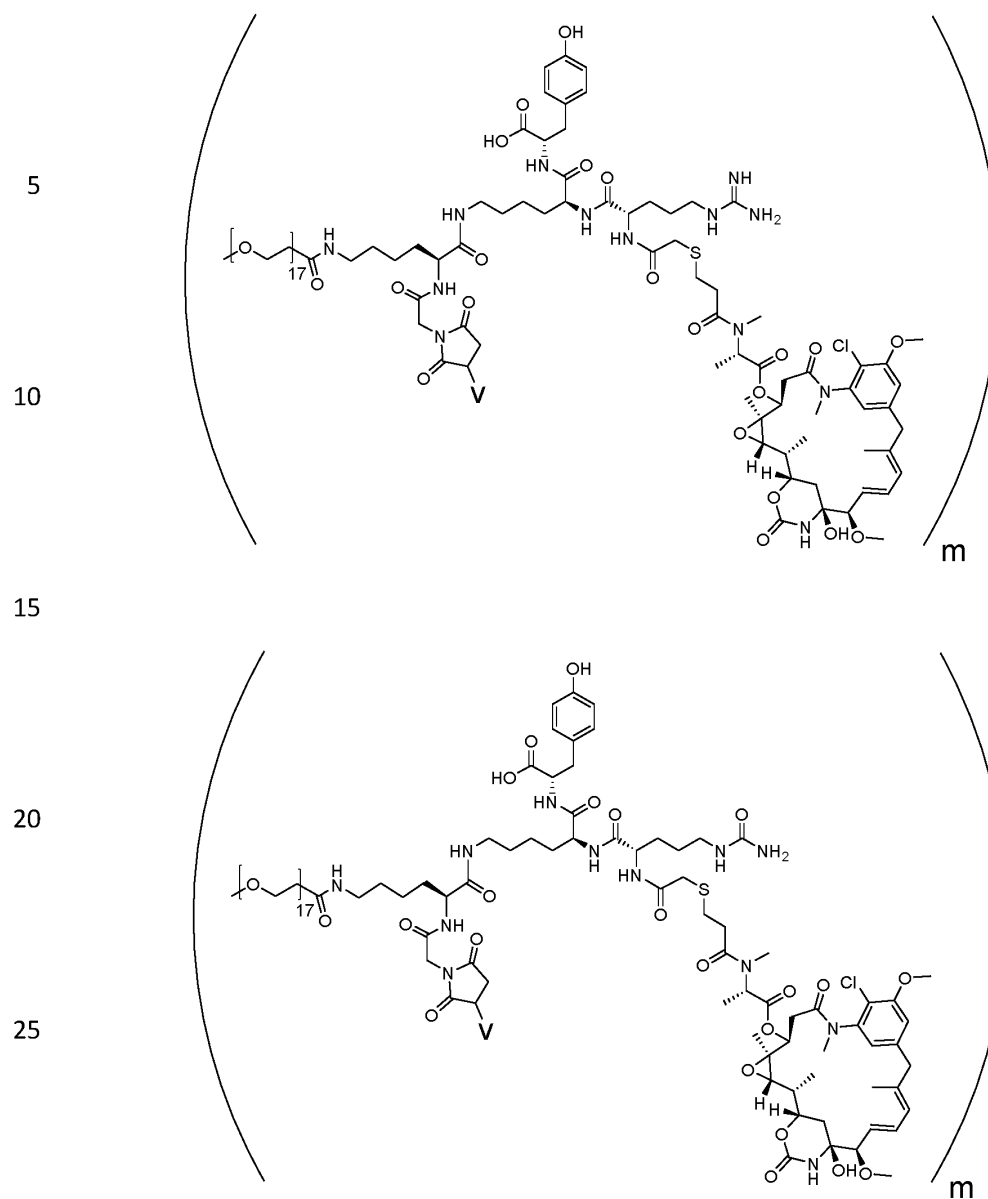
α' indicates covalent attachment to T (to the amino group derived from the Lys residue of T).

In a more preferred embodiment, Y is represented by formula (XIIIb) or (XIIIc), wherein R^3 is preferably a group represented by the formula $-(CH_2)_{n7}-(C=A)_{n9}-$ in which $n7$ is 1 or 2, $n9$ is 1 and A is O. Most preferably, R^3 is $-CH_2-C=O-$.

Further conjugate compounds that may be used in the combinations of the present invention are described in the patent literature, for instance in WO2021087248A1, WO2022235589A1, WO2023068226A1, or US2016051694A.

3.9 Preferred conjugate compounds

In preferred embodiments, (V) represents a moiety derived from an anti-CD37 antibody, preferably naratuximab, and (D) represents a moiety derived from DM1, and the conjugate compound is represented by one of the following formulae:



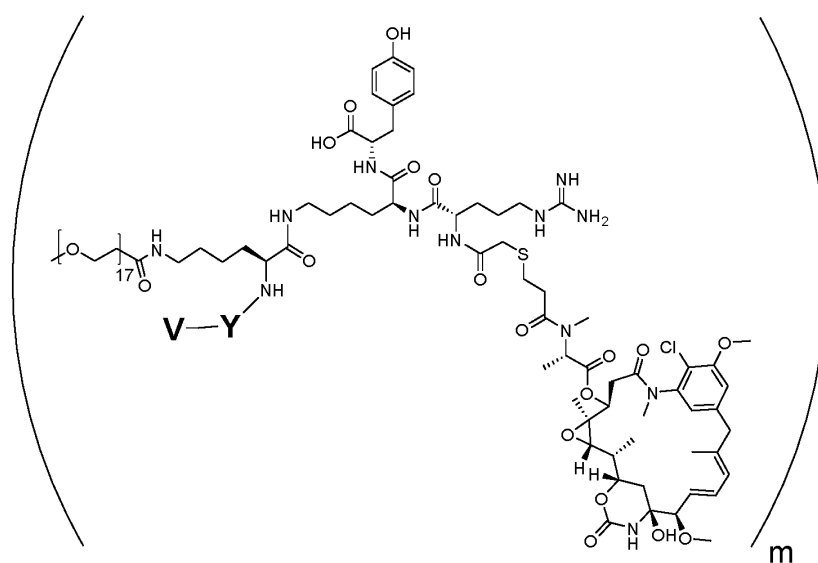
wherein m is an integer of 1 to 12, preferably 2 to 10, more preferably 4 to 8; wherein the number of oxyethylene repeating units (17) may be replaced by 12 to 30, preferably 14 to 25, more preferably 15 to 19 oxyethylene groups; and/or wherein the maleimide attachment to (V) may be replaced by an opened hydrolyzed maleimide.

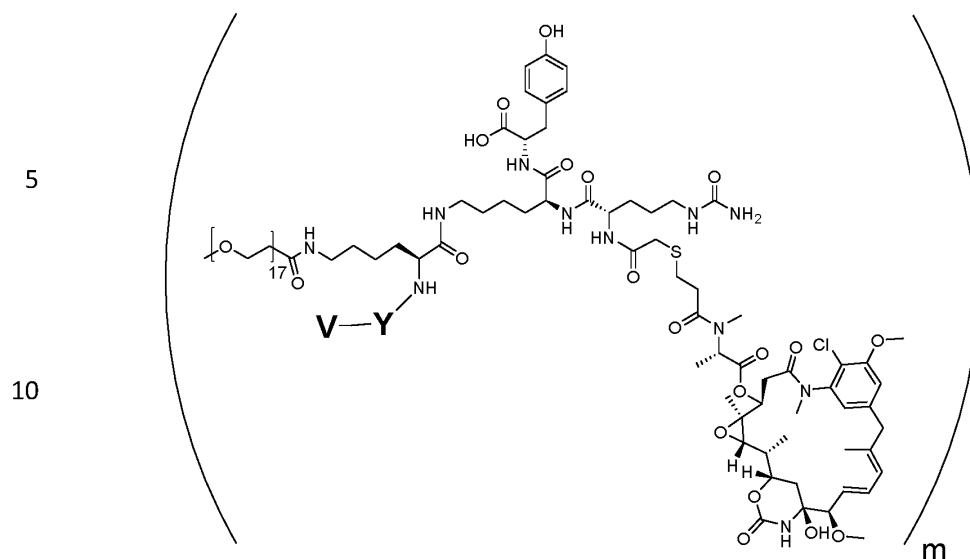
As is evident from formula (I), in the above compounds, the group (V) is to be understood as being outside the parentheses so that indicia m does not apply to (V)

whereas all other depicted structural elements are to be understood as being within the parentheses and thus present in the molecule m times.

According to one embodiment, in the above compounds the maleimide attachment to (V) may be replaced by an opened hydrolyzed maleimide attachment. In some instances, wherein m is at least 2, said compound may comprise a mix of (closed) maleimide derivatives and opened hydrolyzed maleimide derivatives attached to V, preferably at least 50% of the attachments to V are opened hydrolyzed maleimide attachments.

In yet another preferred embodiment, (V) represents a moiety derived from an anti-CD37 antibody, preferably naratuximab, and (D) represents a moiety derived from DM1, and the compound is represented by one of the following formulae:





15 wherein m is an integer of 1 to 12, preferably 2 to 10, more preferably 4 to 8;
wherein Y is represented by any of the formulae (XIIIa) to (XIIIc), preferably by
formula (XIIIb) or (XIIIc); and/or wherein the number of oxyethylene repeating
units (17) may be replaced by 12 to 30, preferably 14 to 25, more preferably 15
20 to 19 oxyethylene groups.

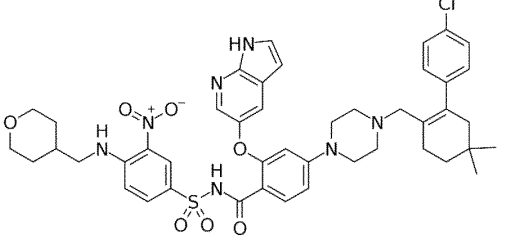
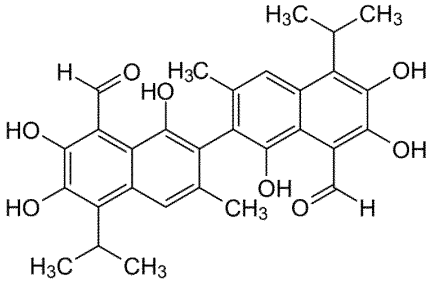
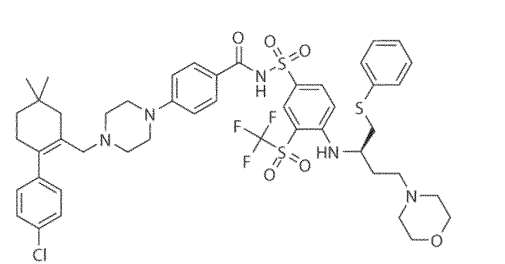
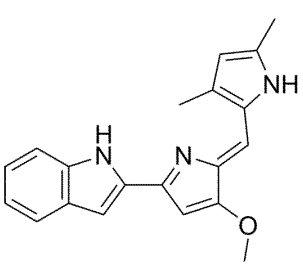
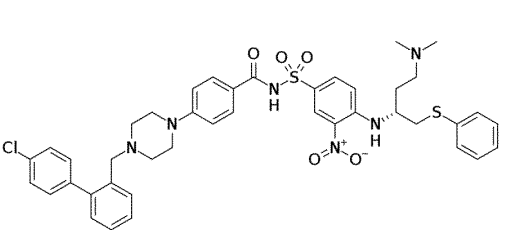
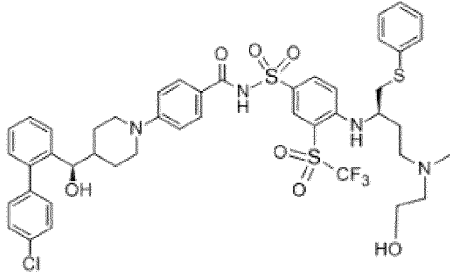
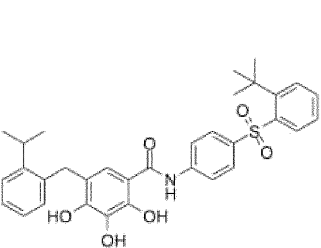
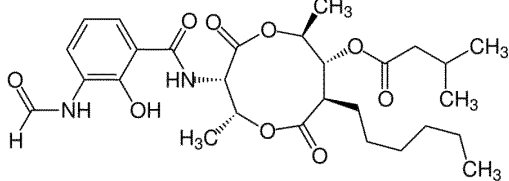
4. The BCL2 inhibitor

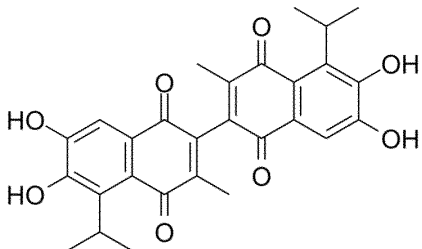
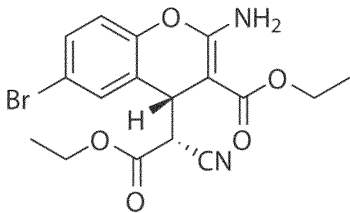
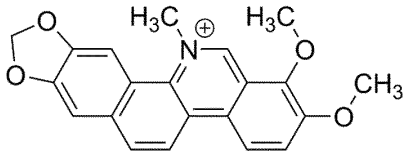
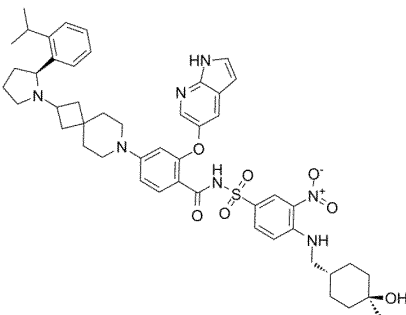
25 BCL2 is a protein with antiapoptotic activity. It is upregulated in many cancers and may therefore serve as a target in cancer therapy. Among the BCL2-inhibiting compounds, one may distinguish between BCL2-selective inhibitors and BCL2 inhibitors binding also to other anti-apoptotic proteins such as BCL-XL and BCL-W.

30 In the context of the present invention, it is possible to use selective or non-selective BCL2 inhibitors. A selective BCL2 inhibitor is for example venetoclax. Non-selective BCL2 inhibitors are for example gossypol, obatoclax, ABT-737, and navitoclax (ABT-263).

35 The list of BCL2 inhibitors suitable for use in the present invention thus includes venetoclax (ABT-199), gossypol, sonrotoclax (BGB 11417), navitoclax (ABT-263), obatoclax (GX15-070), obatoclax mesylate, ABT-737, AZD4320, AZD0466, which is a conjugate of AZD4320 with PEG and a polylysine dendrimer, TW-37, antimycin A, apogossypolone (Apo-G2), HA14-1, and chelerythrine.

The structures of these compounds are shown in the following:

 Chemical structure of Venetoclax, a BCL-2 inhibitor. It features a morpholine ring connected to a benzene ring with a nitro group and a sulfonamide group. The sulfonamide group is linked to another benzene ring, which is further connected to a piperazine ring and a 4-chlorophenyl group. Venetoclax	 Chemical structure of Gossypol, a polyphenolic compound. It consists of two naphthalene units linked by a central carbon-carbon bond. Each naphthalene unit has multiple hydroxyl and aldehyde groups, and is substituted with isopropyl groups. Gossypol
 Chemical structure of Navitoclax, a BCL-2 inhibitor. It features a morpholine ring connected to a benzene ring with a sulfonamide group. The sulfonamide group is linked to another benzene ring, which is further connected to a piperazine ring and a 4-chlorophenyl group. Navitoclax	 Chemical structure of Obatoclax, a BCL-2 inhibitor. It features a benzimidazole core with a methoxy group and a methyl group. Obatoclax
 Chemical structure of ABT-737, a BCL-2 inhibitor. It features a morpholine ring connected to a benzene ring with a sulfonamide group. The sulfonamide group is linked to another benzene ring, which is further connected to a piperazine ring and a 4-chlorophenyl group. ABT-737	 Chemical structure of AZD4320, a BCL-2 inhibitor. It features a morpholine ring connected to a benzene ring with a sulfonamide group. The sulfonamide group is linked to another benzene ring, which is further connected to a piperazine ring and a 4-chlorophenyl group. AZD4320
 Chemical structure of TW-37, a BCL-2 inhibitor. It features a morpholine ring connected to a benzene ring with a sulfonamide group. The sulfonamide group is linked to another benzene ring, which is further connected to a piperazine ring and a 4-chlorophenyl group. TW-37	 Chemical structure of Antimycin A, a polyketide antibiotic. It features a complex polycyclic core with multiple hydroxyl and carbonyl groups, and is substituted with methyl and ethyl groups. Antimycin A

 Apogossypolone	 HA14-1
 Chelerythrine	 Sonrotoclax

It is also possible to use other BCL2 inhibitors that are not described above in the context of the present invention. For instance, further BCL2 inhibitors suitable for use in the combinations of the present invention are described in Roberts AW, Wei AH, Huang DCS. BCL2 and MCL1 inhibitors for hematologic malignancies. Blood. 2021 Sep 30;138(13):1120-1136. doi: 10.1182/blood.2020006785 and in Suvana V, Singh V, Murahari M. Current overview on the clinical update of Bcl-2 anti-apoptotic inhibitors for cancer therapy. Eur J Pharmacol. 2019 Nov 5;862:172655. doi: 10.1016/j.ejphar.2019.172655. Among the BCL2 inhibitors described above, venetoclax, navitoclax and ABT-737 are preferred.

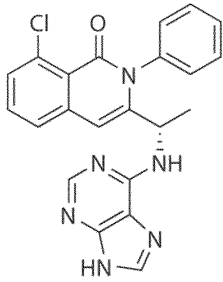
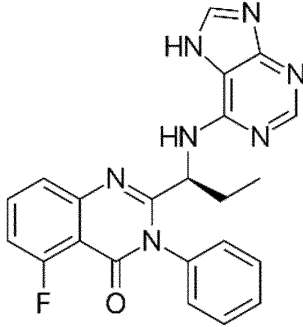
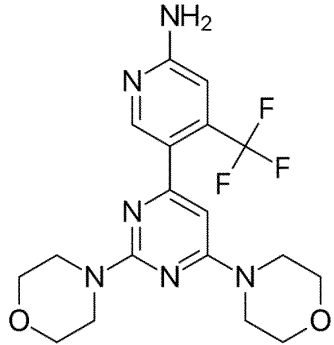
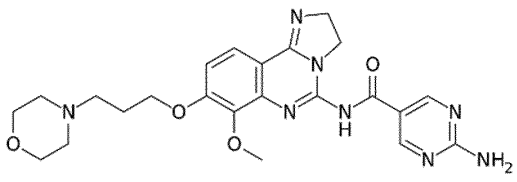
5. The PI3K pathway inhibitor

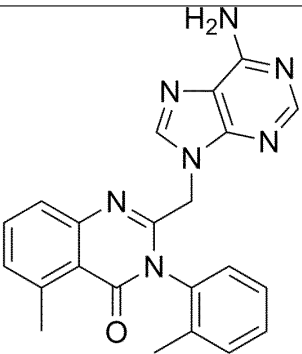
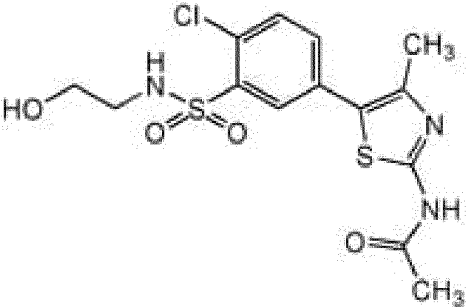
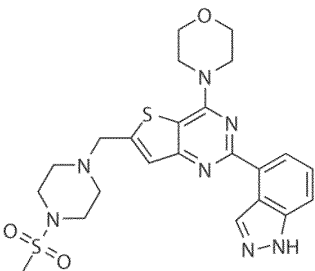
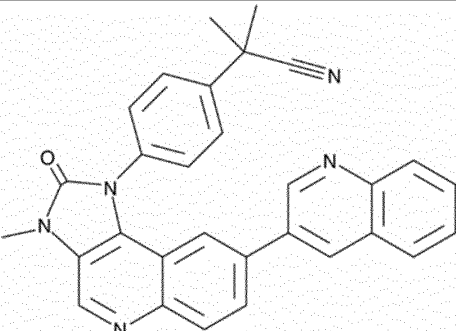
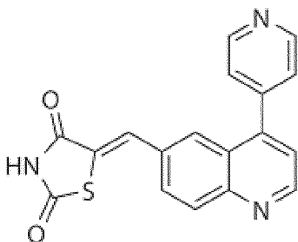
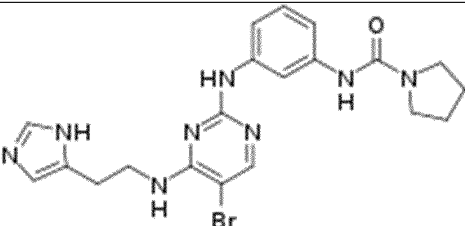
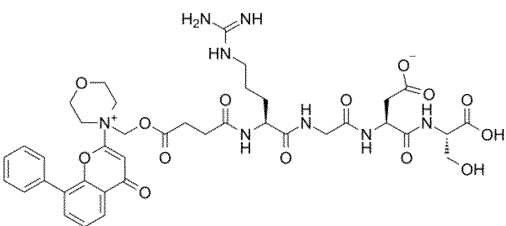
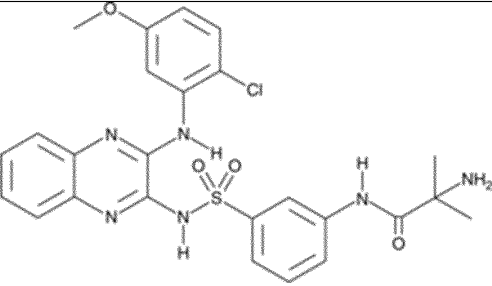
The PI3K pathway comprises the members phosphoinositide 3-kinase (PI3K), protein kinase B (Akt) and mammalian target of rapamycin (mTOR). Inhibition of the PI3K pathway may be accomplished at any stage of the pathway. Hence, the PI3K pathway

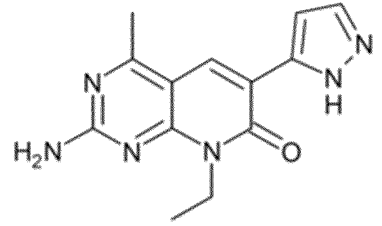
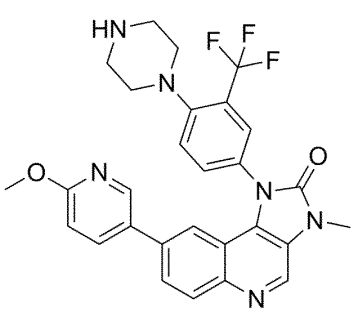
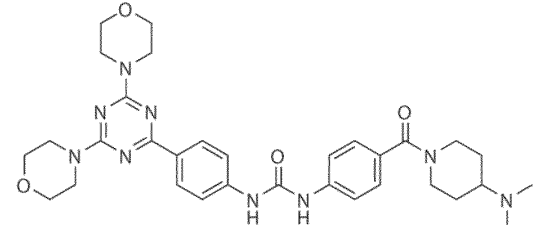
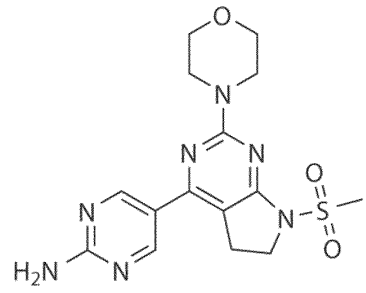
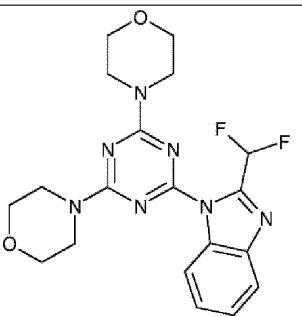
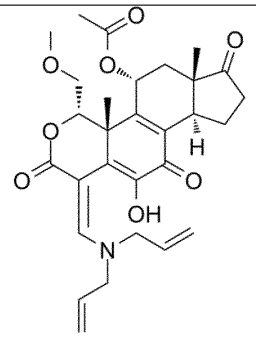
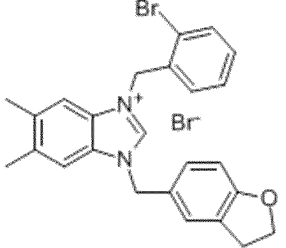
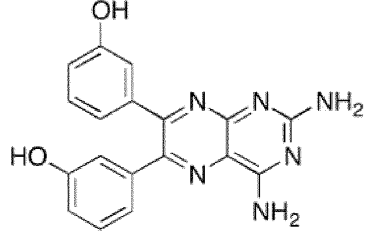
inhibitor suitable for use in the combination of the present invention may be selected from PI3K inhibitors, Akt inhibitors and mTOR inhibitors.

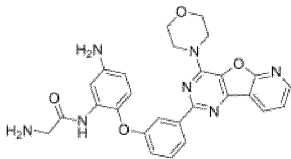
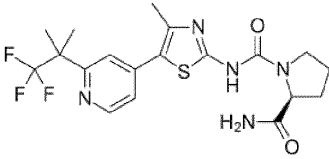
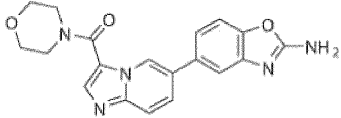
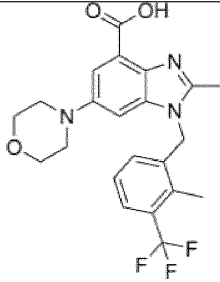
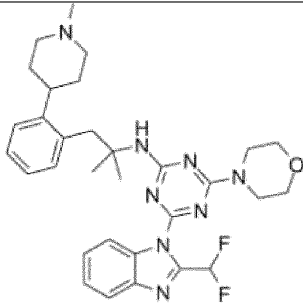
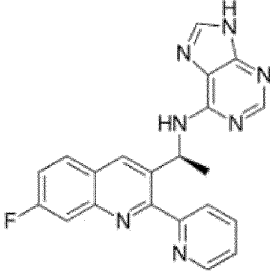
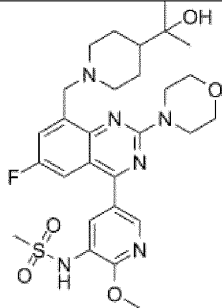
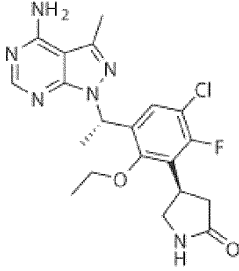
The PI3K inhibitor may for instance be selected from duvelisib (IPI-145), idelalisib (CAL-101), buparlisib (AN2025), roginolisib (MSC2360844), copanlisib (BAY 80-6946), IC-87114, PIK-93, pictilisib (GDC-0941), dactolisib (BEZ235), GSK1059615, BX-912, SF1126, pilarsilib (SAR245408), voxtalisib (SAR245409), BGT226, gedatolisib (PKI-587), NVPBE235, izorlisib (CH5132799), ZSTK474, sonolisib (PX-866), B591, TG100-115, RIDR-PI-103, alpelisib (BYL719), serabelisib (INK-117), GSK2636771, zandelisib (ME-401), AMG319, linperlisib (YY-20394), parsaclisib (INCB050465), umbralisib (TGR-1202), PF-04691502, tenalisib (RP6530), taseclisib (GDC-0032), AZD8186, AZD8835, duvelisib (IPI-145), leniolisib (CDZ173), eganelisib (IPI-549), apitolisib (GDC-0980), omipalisib (GSK2126458), samotolisib (LY3023414), bimiralisib (PQR309), paxalisib (GDC-0084), voxtalisib (XL765), SAR245409, VS-5584.

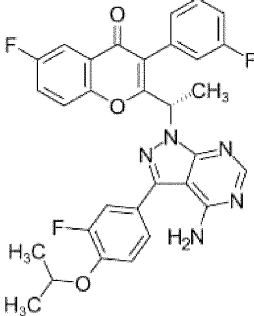
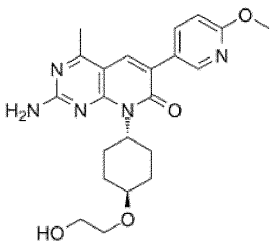
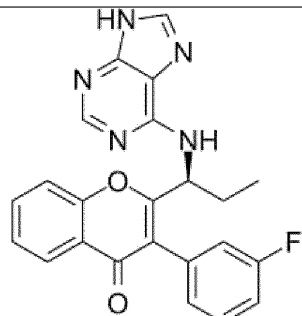
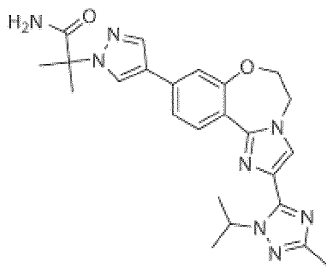
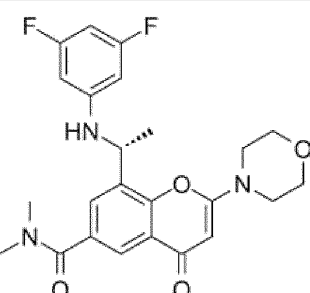
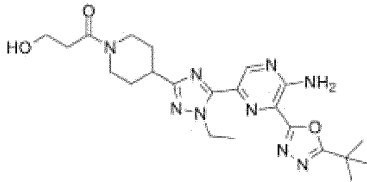
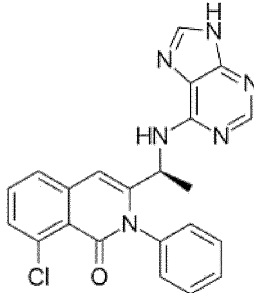
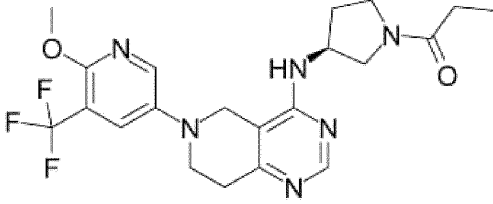
The structures of these compounds are shown in the following:

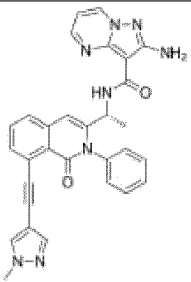
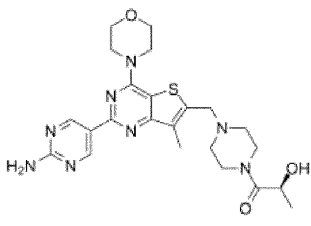
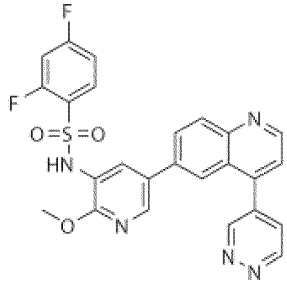
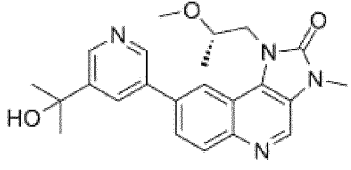
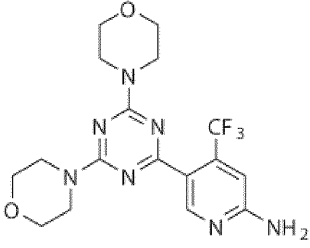
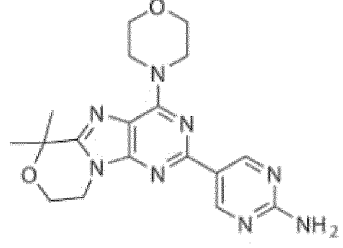
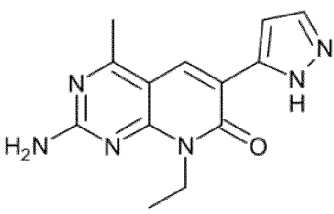
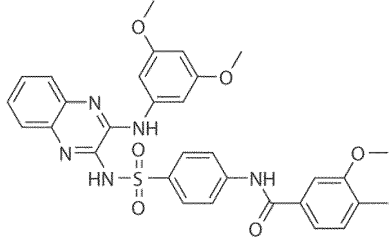
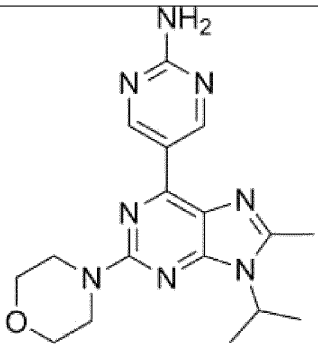
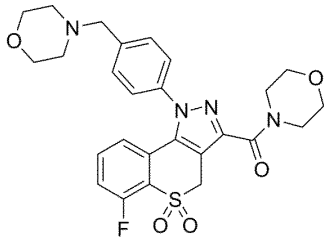
 Duvelisib	 Idelalisib
 Buparlisib	 Copanlisib

 IC-87114	 PIK-93
 Pictilisib	 Dactolisib
 GSK1059615	 BX-912
 SF1126	 Pilarsilib

 Voxtalisib	 BGT226
 PKI-587	 Izorlisib
 ZSTK474	 Sonolisib
 B591	 TG100-115

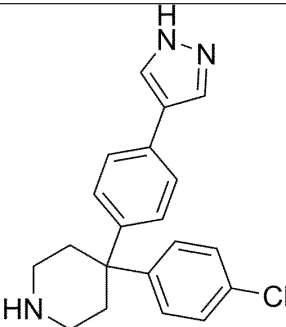
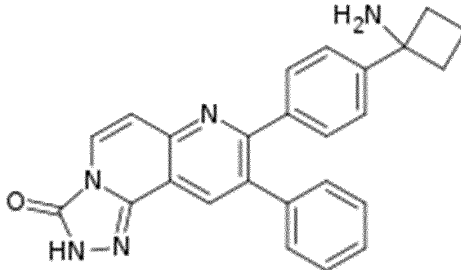
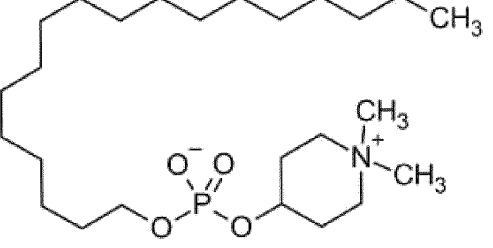
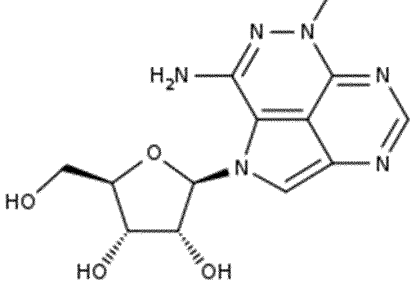
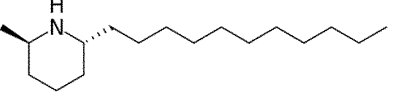
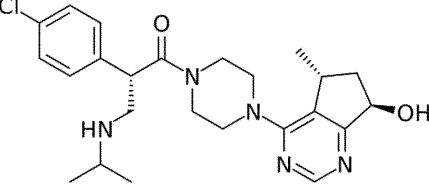
 RIDR-PI-103	 Alpelisib
 Serabelisib	 GSK2636771
 Zandelisib	 AMG-319
 Linperlisib	 Parsaclisib

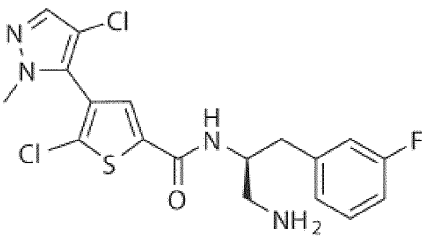
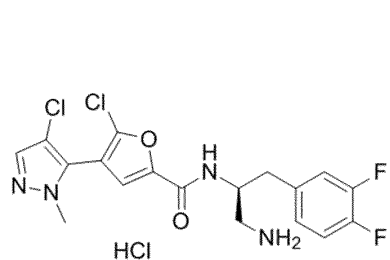
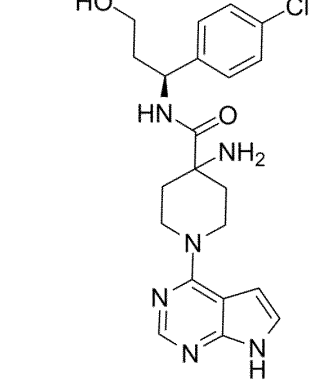
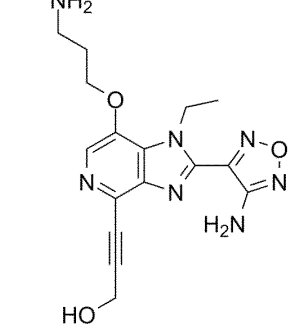
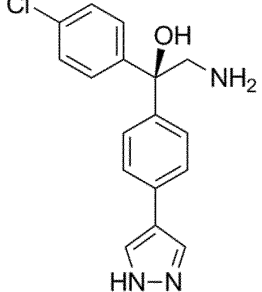
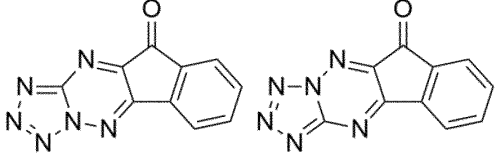
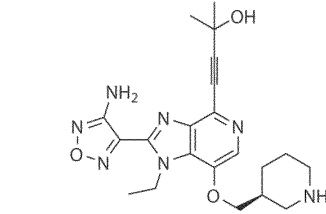
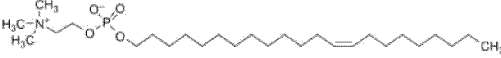
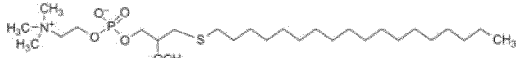
 Umbralisib	 PF-04691502
 Tenalisib	 Taselisib
 AZD8186	 AZD8835
 Duvelisib	 Leniolisib

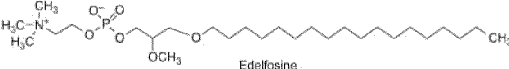
 Eganelisib	 Apitolisib
 Omipalisib	 Samotolisib
 Bimiralisib	 Paxalisib
 Voxtalisib	 SAR245409
 VS-5584	 Roginolisib

The Akt inhibitor may for instance be selected from AT7867, MK-2206, perifosine (KRX-0401), triciribine, ipatasertib (RG7440), afuresertib (GSK2110183), uprosertib (GSK2141795), capivasertib (AZD5363), solenopsin, solenopsin analogues, HY-10249A, AT13148, KP372-1, GSK690693, erufosine, erucylphosphocholine, ilmofofosine, edelfosine.

The structures of these compounds are shown in the following:

 AT7867	 MK-2206
 Perifosine	 Triciribine
 Solenopsin	 Ipatasertib

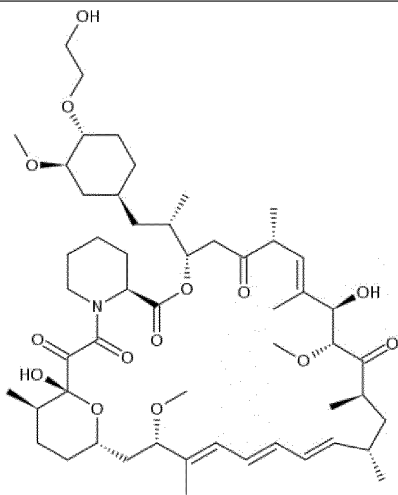
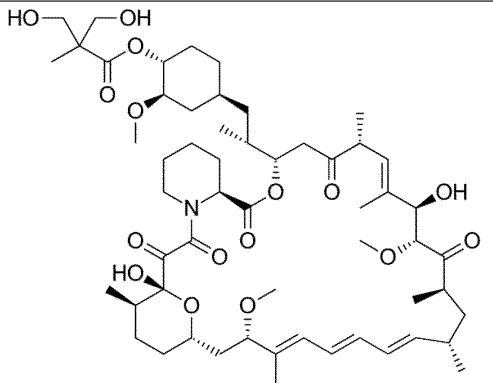
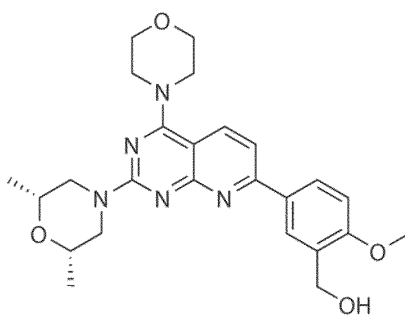
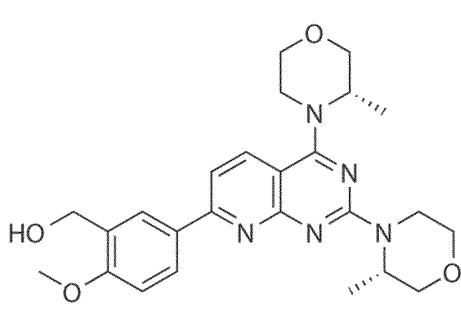
 Afuresertib	 Uprosertib
 Capivasertib	 HY-10249A
 AT13148	 KP372-1
 GSK690693	 Erufosine
 GSK690693	 Ilimofosine

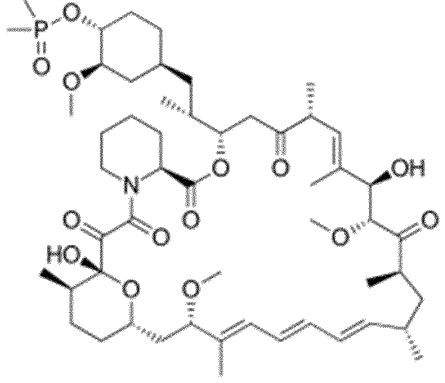
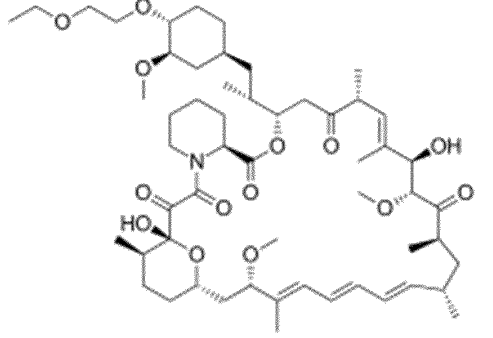
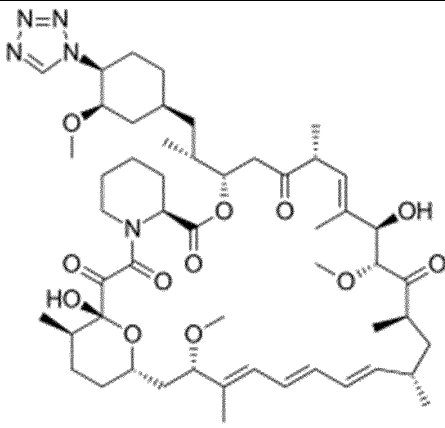
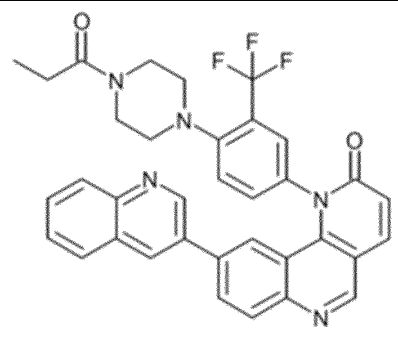
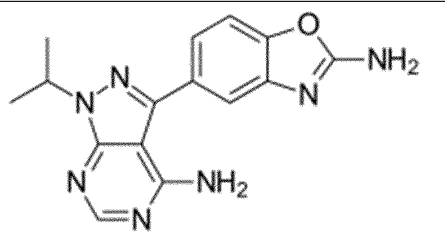
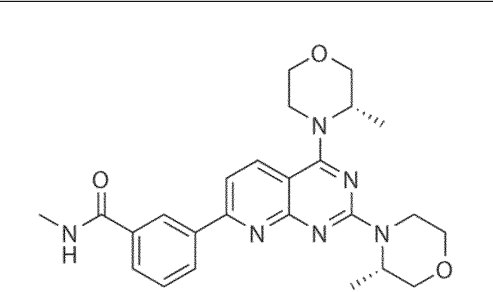
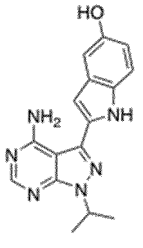
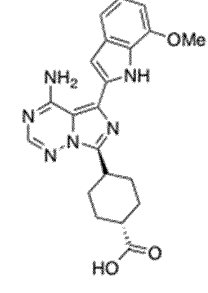
Erucylphosphocholine	Ilmofofosine
 Edelfosine	

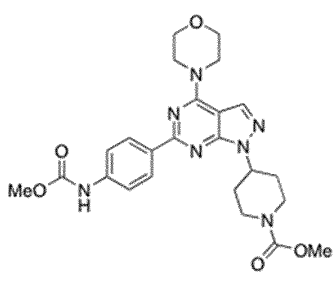
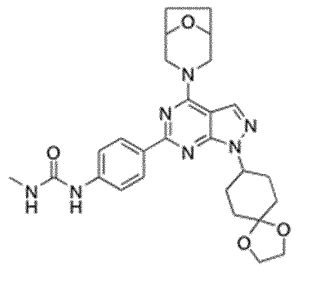
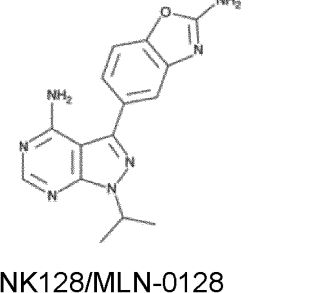
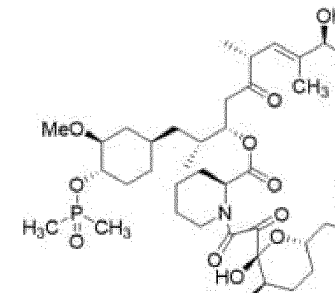
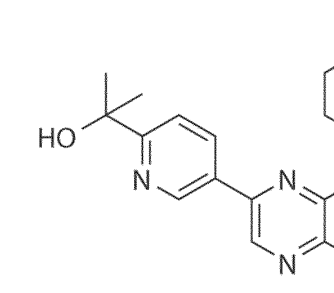
The mTOR inhibitor may for instance be selected from everolimus, temsirolimus, KU 0063794, AZD 8055, ridaforolimus (AP23573), umirolimus, zotarolimus, torin-1, sapanisertib (INK128), vistusertib (AZD2014), PP242, OSI-027, WYE354, WYR-125132, INK128/MLN-0128, deforolimus, onatasertib (CC-223), as well as the dual PI3K/mTOR inhibitors such as dactolisib, pilarlisib, voxalisib, apitolisib, gedatolisib, BGT226, GSK2126458, PF-04691502, VS-5584, SF-1126.

The structures of these compounds are shown in the following:

10

 Everolimus	 Temsirolimus
 KU0063794	 AZD8055

 Chemical structure of Ridaforolimus, a macrocyclic rapamycin derivative. It features a large 26-membered macrocycle with multiple ester, ketone, and hydroxyl functional groups, and a side chain containing a long polyunsaturated fatty acid moiety.	 Chemical structure of Umirolimus, a macrocyclic rapamycin derivative. It features a large 26-membered macrocycle with multiple ester, ketone, and hydroxyl functional groups, and a side chain containing a long polyunsaturated fatty acid moiety. Umirolimus
 Chemical structure of Zotarolimus, a macrocyclic rapamycin derivative. It features a large 26-membered macrocycle with multiple ester, ketone, and hydroxyl functional groups, and a side chain containing a long polyunsaturated fatty acid moiety. Zotarolimus	 Chemical structure of Torin-1, a mTOR inhibitor. It consists of a quinoline core substituted with a 4-ethylpiperazine-1-yl group, a 2,2,2-trifluoroethyl group, and a 4-quinolinecarboxamido group. Torin-1
 Chemical structure of Sapanisertib, a JAK inhibitor. It features a pyrazolo[1,5-a]pyrimidine core substituted with an isopropyl group, an amino group, and a 4-amino-2-oxo-1,2,3,4-tetrahydrobenzoxazole-5-yl group. Sapanisertib	 Chemical structure of Vistusertib, a JAK inhibitor. It features a pyrimidine core substituted with a 4-(methylamino)benzoyl group, a 4-(methylamino)benzoyl group, and a 4-(methylamino)benzoyl group. Vistusertib
 Chemical structure of PP242, a JAK inhibitor. It features a pyrazolo[1,5-a]pyrimidine core substituted with an isopropyl group, an amino group, and a 4-amino-2-oxo-1,2,3,4-tetrahydrobenzoxazole-5-yl group. PP242	 Chemical structure of OSI-027, a JAK inhibitor. It features a pyrazolo[1,5-a]pyrimidine core substituted with an isopropyl group, an amino group, and a 4-amino-2-oxo-1,2,3,4-tetrahydrobenzoxazole-5-yl group. OSI-027

 WYE354	 WYE-125132
 INK128/MLN-0128	 Deforolimus
 CC-223	

Alternative inhibitors of the PI3K pathway not shown above may also be used in the combinations of the present invention. For instance, mTOR inhibitors suitable for use in the combinations of the present invention are described in Mao B, Zhang Q, Ma L, Zhao DS, Zhao P, Yan P. Overview of Research into mTOR Inhibitors. *Molecules*. 2022 Aug 19;27(16):5295. doi: 10.3390/molecules27165295.

Among the PI3K inhibitors listed above, IPI-145, CAL-101 (idelalisib), IC-87114, PIK-93, GDC-0941, GSK1059615, KU0063794, AZD8055, MK-2206 and triciribine are preferred.

6. Combinations

The combinations of the present invention comprise at least the conjugate compound of formula (I) described herein, together with a BCL2 inhibitor or an inhibitor of the PI3K pathway (such as a PI3K inhibitor, an Akt inhibitor or an mTOR inhibitor). These inhibitors are jointly referred to as co-agents. The conjugate compound and the co-agent may be administered at the same time, at approximately the same time, the conjugate compound may be administered prior to the co-agent or the co-agent may be administered prior to the conjugate compound. By consequence, the conjugate compound and the co-agent may be provided as part of the same pharmaceutical composition or they may be provided in the form of separate pharmaceutical compositions, for instance in the form of a kit comprising said separate pharmaceutical compositions in separate containers.

The molar ratio of the conjugate compound to the co-agent is not particularly restricted and may vary for instance from $1:10^4$ to $10^4:1$. Typically, however, the conjugate compound is administered in smaller amounts than the co-agent, so that the molar ratio of the conjugate compound to the co-agent may range from $1:10^4$ to $1:1$.

7. Pharmaceutical compositions

The compounds of the present invention can be provided in the form of pharmaceutical compositions for human or animal usage in human and veterinary medicine. Such compositions typically comprise a therapeutically effective amount of the conjugate compound and/or the co-agent or a pharmaceutically acceptable salt thereof, and one or more components selected from a carrier, a diluent and other excipients.

In one embodiment, the pharmaceutical composition comprises a mixture of multiple conjugate compounds and/or a mixture of multiple co-agents.

In a more specific embodiment, the pharmaceutical composition comprises a mixture of conjugate compounds wherein said conjugate compounds (compounds of formula I) comprise (closed) maleimide and/or open hydrolysed maleimide attachments to V (as described above).

The proportions of (closed) maleimide derivatives (A) and opened hydrolyzed maleimide derivatives (B) attached to V (in terms of total maleimide attachments in the composition) may be A : B / 0-50 : 50-100 %, preferably A : B / 10-40 : 60-90 %, more

preferably A : B / 15-35 : 65-85 %, and most preferably about A : B / 30 : 70 %.

The respective proportions of closed maleimide derivatives and opened hydrolyzed maleimide derivatives (and thereby closed (A) and open (B) maleimide attachments)
5 in a composition can be determined by MS techniques such as Tof or Orbitrap analysis of the reduced LDC (Compounds of formula (I)). An example of detailed protocol is available in chapter 6.d. of Chem. Eur. J. 2019, 25, 8208-8213.

Suitable carriers, diluents and other excipients for use in pharmaceutical compositions
10 are well known in the art, and are for instance described in Remington's Pharmaceutical Sciences, Mack Publishing Co. (Gennaro AR, 1985). The carrier, diluent and/or other excipient can be selected with regard to the intended route of administration and pharmaceutical practice. The pharmaceutical compositions can comprise as the carrier, diluents and/or other excipients, or in addition to, any suitable
15 binder(s), lubricant(s), suspending agent(s), coating agent(s), solubilizing agent(s).

The therapeutically effective amount can be determined by a physician on a routine basis. The specific dose level and frequency of dosage for any particular subject/patient can vary and depends on a variety of factors including the activity of
20 the specific drug compounds employed, the metabolic stability and length of action of that compounds, their mixing ratio, the patient's age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the individual undergoing therapy. These factors are taken into account by the physician when determining the therapeutically affective
25 dose.

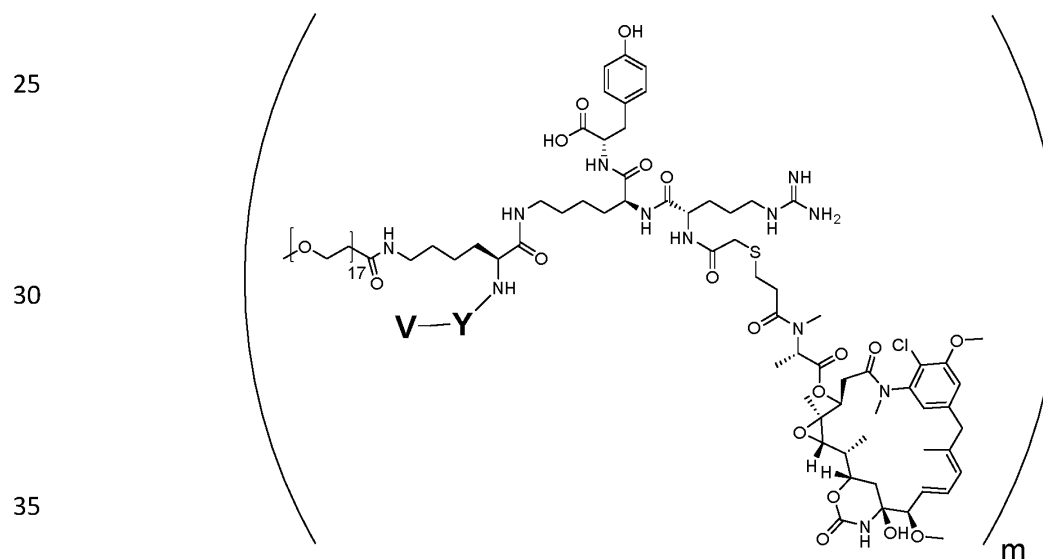
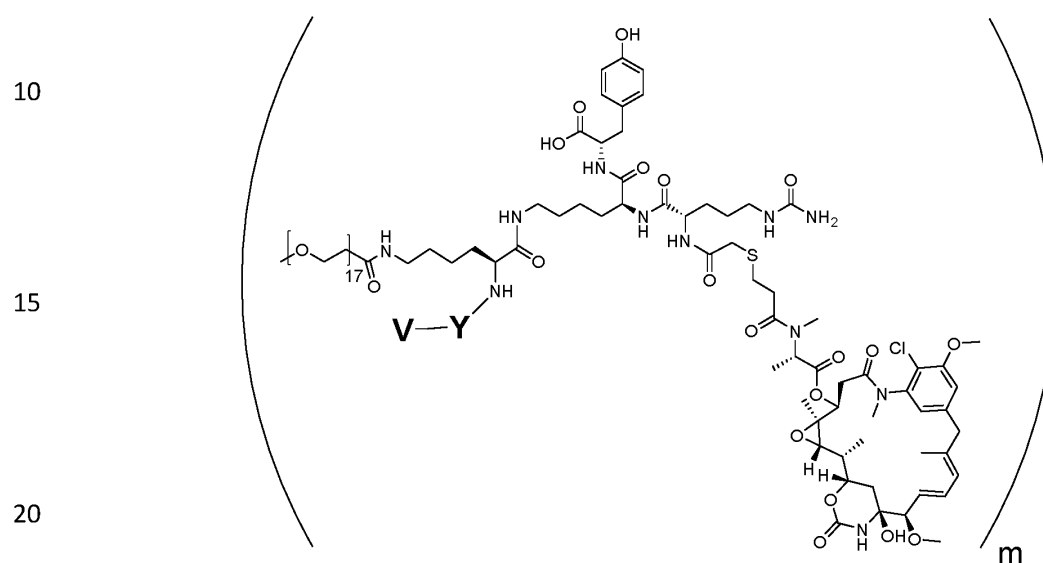
8. Use of combination in methods of preventing or treating diseases

The compounds of the present invention can be used to treat cancer and especially
30 DLBCL. The treatment can be a therapeutic and/or prophylactic treatment, with the aim being to prevent, reduce or stop an undesired physiological change or disorder. In some aspects, the treatment can prolong survival of a subject as compared to expected survival if not receiving the treatment.

35 In some embodiments, the combination of the present invention is used in a method of treating or preventing DLBCL.

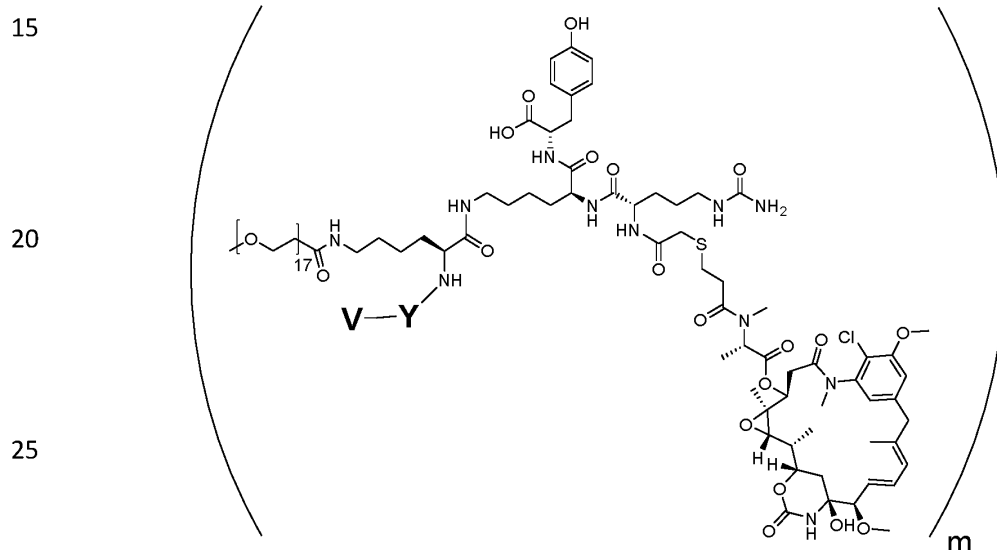
In a preferred embodiment, the combination of the present invention or composition thereof is used in a method of treating or preventing DLBCL, said combination

comprising a BCL2 inhibitor, preferably venetoclax, or a PI3K pathway inhibitor, preferably idelalisib, together with a conjugate compound as shown below, wherein (V) represents a moiety derived from an anti-CD37 antibody, preferably naratuximab, (D) represents a moiety derived from DM1, and the compound is represented by one of the following formulae:



wherein m is an integer of 1 to 12, preferably 2 to 10, more preferably 4 to 8;
 wherein Y is represented by any of the formulae (XIIIa) to (XIIIc), preferably by
 formula (XIIIb) or (XIIIc); and/or wherein the number of oxyethylene repeating
 units (17) may be replaced by 12 to 30, preferably 14 to 25, more preferably 15
 5 to 19 oxyethylene groups.

In a more preferred embodiment, the combination of the present invention or
 composition thereof is used in a method of treating or preventing DLBCL, said
 combination comprising a BCL2 inhibitor, preferably venetoclax, or a PI3K pathway
 10 inhibitor, preferably idelalisib, together with a conjugate compound as shown below,
 wherein (V) represents a moiety derived from an anti-CD37 antibody, preferably
 naratuximab, (D) represents a moiety derived from DM1, and the conjugate compound
 is represented by the following formula:



wherein m is an integer of 1 to 12, preferably 2 to 10, more preferably 4 to 8;
 wherein Y is represented by any of the formulae (XIIIa) to (XIIIc), preferably by
 formula (XIIIb) or (XIIIc); and/or wherein the number of oxyethylene repeating
 units (17) may be replaced by 12 to 30, preferably 14 to 25, more preferably 15
 30 to 19 oxyethylene groups.

35 As evident from formula (I), in the above compounds, the group (V) is to be understood
 as being outside the parentheses so that indicia m does not apply to (V) whereas all
 other depicted structural elements are to be understood as being within the
 parentheses and thus present in the molecule m times.

The combination of the invention may be administered to a subject (e.g., a patient) in any therapeutically effective dose. Said therapeutically effective dose may depend on the characteristics of the patient in question e.g., size and weight.

- 5 The conjugate compound can be administered to a subject (e.g. a patient) at one time or over a series of treatments. Depending on the type and severity of the disease, between about 0.1 µg/kg to 1 mg/kg of drug may be used as an initial candidate dosage for first administration in a first-in-human trial, e.g. by one or more separate administrations, or by continuous infusion. A typical daily, once weekly (QW), once
10 every two weeks (Q2W), once every 3 weeks (Q3W) or monthly dosage can range from about 0.1 mg/kg to 50 mg/kg or more, or from about 0.5 to about 25 mg/kg of patient weight. The subject to whom the compound/molecule is administered may be a patient in need thereof i.e., a DLBCL patient.
- 15 In the combinations of the invention, the BCL2 inhibitor and the PI3K pathway inhibitor may be administered as per indicated clinical posology, such as in a DLBCL clinical trial or an approved market authorization for DLBCL patients.

- The combination of the invention may also be administered to a patient suffering from
20 a DLBCL cancer, wherein the DLBCL is resistant to monotherapy treatment with a conjugate compound of formula (I), or as further defined herein. In some aspects of such a resistant DLBCL, the treatment may include, for example, pretreatment with a PI3K pathway inhibitor, preferably a PI3Kδ inhibitor such as idelalisib, followed by treatment with a conjugate compound of formula (I), or followed by concurrent
25 administration of both agents of the combination of the invention, i.e. the conjugate compound of formula (I) and the co-agent.

- When treating cancer, the therapeutically effect that is observed can be a reduction in the number of cancer cells; a reduction in tumor size; inhibition or retardation of cancer
30 cell infiltration into peripheral organs; inhibition of tumor growth; and/or relief of one or more of the symptoms associated with cancer.

- The routes for administration (delivery) include one or more of oral (e.g. tablet, capsule, ingestible solution), topical, mucosal (e.g. nasal spray, aerosol for inhalation), nasal,
35 parenteral (e.g. an injectable form), gastrointestinal, intraspinal, intraperitoneal, intramuscular, intravenous, intrauterine, intraocular, intradermal, intracranial, intratracheal, intravaginal, intracerebroventricular, intracerebral, subcutaneous, ophthalmic (including intravitreal or intracameral), transdermal, rectal, buccal, vaginal,

epidural, sublingual. According to a preferred embodiment, the compound of the present invention is administered by injection, such as parenterally, intravenously, subcutaneously, intramuscularly, transdermally.

5 9. Preparation of the compounds of the invention

In the following, methods are provided for the preparation of linkers, drug-linkers and ligand-drug-conjugates. The compounds of the invention can be synthesized relying on standard organic chemistry reactions or Fmoc-based solid-phase peptide synthesis (SPPS), including in solution and on-resin peptide coupling and convergent strategies. The introduction of various maleimido-derivatives and subsequent chemoselective ligation to moieties derived from a vector group is also exemplified below. The general strategies and methodology which can be used for preparing the compounds of the present invention are well-known to the person skilled in the art.

15

10. Examples

The synthetic and analytical experiments relating to the conjugate compounds alone are based on the disclosure of co-pending application PCT/EP2023/059781. Further experimental details may be found in the examples section of said co-pending application.

20

10.1 List of abbreviations used in the examples:

25 Ac: Acetyl
ADC: Antibody-Drug Conjugate
ACN: acetonitrile
AMAS: N- α -maleimidoacet-oxysuccinimide ester
Arg: Arginine
30 Bn: benzyl
Boc: tert-butyloxycarbonyl
Bu: butyl
Cit: Citrulline
DAR: Drug to Antibody Ratio
35 DBU: 1,8-Diazabicyclo[5.4.0]undéc-7-ène
DCM: dichloromethane
DIEA: diisopropylethylamine
DLBCL: Diffuse large B cell lymphoma

- DM1: Mertansine
DMF: dimethyl formamide
DMSO: dimethyl sulfoxide
DOC: degree of conjugation
- 5 DPBS: Dulbecco's phosphate-buffered saline
EDC: 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
Et: ethyl
eq.: equivalent
FA: formic acid
- 10 Fmoc: 9-Fluorenylmethoxycarbonyl
g: gram
Gly: Glycine
Glu: Glutamic acid
h: hour
- 15 HATU: 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide
HPLC: high-performance liquid chromatography
HRMS: high resolution mass spectrometry
IR: Infrared
L: litre
- 20 Lys: Lysine
m: milli
ma: maleimidoacetic acid
mAb: monoclonal antibody
Me: methyl
- 25 min: minute
Mtt: 4-methyltrityl
mol: molar
MS: mass spectroscopy
m/z: ratio mass over charge
- 30 NHS: N-hydroxysuccinimide
Nmab: Naratuximab
Pbf: 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl
PBS: phosphate-buffered saline
PEG: polyethylene glycol
- 35 PEG16 or Peg16: CO-CH₂CH₂O-(CH₂CH₂O)₁₆-CH₃
PF: CentriPure PF filtration columns
PFP: pentafluorophenyl
pH: potential for hydrogen

- Phe: Phenylalanine
quant.: quantitative
rt: room temperature
Rt: retention time
- 5 SMCC: Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate
Suc : succinic
TCEP: tris(2-carboxyethyl)phosphine
TFA: trifluoroacetic acid
Tmab: Trastuzumab
- 10 Tyr: Tyrosine
UPLC: ultra-performance liquid chromatography
UV: ultraviolet
 μ : micro

15 **10.2 Starting materials and chemicals:**

The main starting materials and chemicals used in the following examples are listed below:

- > Solvents for synthesis and deprotection reagents from Merck or Fischer Scientific AG (Switzerland);
- 20 > TFA, DIEA, N-hydroxysuccinimide, DBU, DPBS, 10x DPBS and H-Glu(OtBu)-OBn from Sigma-Aldrich (Switzerland);
- > Fmoc-Cit-OPFP, H-Lys(Boc)-OH, Fmoc-Glu(OtBu)-OH, H-Tyr(OtBu)-OtBu, Fmoc-Lys(Mtt)-OH, Fmoc-Cit-OH, Fmoc-Phe-OH and Boc-Cit-OH from Bachem
- 25 (Switzerland);
- > AMAS from Astatech Inc (USA);
- > Solvents and chemicals for high-performance liquid chromatography (HPLC) and ultra-performance liquid chromatography mass spectrometry (UPLC-MS) from Biosolve (France);
- 30 > PEG24-amine from BroadPharm (USA);
- > HATU from Combi-Blocks (USA);
- > Exatecan mesylate from Angel Pharmatech, Ltd. (China);
- > LiOH.H₂O from VWR (Switzerland);
- > DM1-SMCC from Enovation Chemicals (USA);
- 35 > Boc-Gly-OH and dihydrofuran-2,5-dione from Fluka (USA);
- > EDC.HCl from Apollo Chemical (USA);
- > DM1 from Angel Pharmatech, Ltd. (USA);
- > TCEP.HCl, Boc-Lys-OH from Fluorochem (UK);

- > BrCH₂ COOH, H-Tyr-OMe and Pd/C from Acros Organics (Belgium);
- > H-Cit-Lys(PEG5-ma)-Tyr-OH from AmbioPharm (USA)
- > Herceptin® (trastuzumab) from Roche (Switzerland);
- > Naratuximab - antibody huCD37-3 (version 1.0) described in WO2019/229677;
- 5 > mPEG16-NHS ester from PurePEG (mPEG₁₆-OCH₂CH₂COO-NHS ester, m=methyl), LLC (USA);
- > N-succinimidyl-4-(maleimidomethyl)-cyclohexanecarboxylate (SMCC) from Sigma Aldrich Fine Chemicals
- > BCL2 inhibitors and PI3K pathway inhibitors used in the experiments were
- 10 obtained from commercial suppliers.

10.3 Analytical Methods:

The following methods were used to evaluate the compounds and conjugates of the
15 present invention:

10.3.1 Purity determination

The purity of the compounds was determined on UPLC-MS systems:
20

- Method 1: Waters Acquity UPLC System coupled to a Waters SQD mass spectrometer with a CSH C18 column (130 Å, 1.7 µm, 2.1 mm x 50 mm) heated at 40 °C using solvent systems A (water+0.1% FA) and B (ACN+0.1% FA) at a flow rate of 0.9 mL/min and a 5–100% gradient of B over 2.7 min.
- 25 • Method 2: Waters Acquity UPLC System coupled to a Waters SQD mass spectrometer with a CSH Fluoro-phenyl column (130 Å, 1.7 µm, 2.1 mm x 50 mm) heated at 40 °C using solvent system A (water+0.1% FA) and B (ACN+0.1% FA) at a flow rate of 0.9 mL/min and a 5–100% gradient of B over 2.9 min.
- 30 • Method 3: Waters Acquity UPLC System coupled to a Waters SQD mass spectrometer, BEH C18 1.7µm 50x2.1mm column heated at 40°C and fitted with 2µm insert filter pre-columns (available from Waters), and solvent systems A1 (water+0.1%FA) and B1 (ACN+0.1%FA) at a flow rate of 0.9 mL/min and a 5–100% gradient of B1 over 2.9 min.
- 35 • Method 4: Waters Acquity UPLC System coupled to a Waters SQD mass spectrometer with a CSH C18 column (130 Å, 1.7µm, 2.1 mm x 50 mm) heated at 40°C using solvent system A (water+0.1%FA) and B (ACN+0.1%FA) at a flow rate of 0.6 mL/min and a 5-85% gradient of B over 5 min.

- Method 5: Equipment: Shimadzu LCMS 2020 Mass Spectrometer. Column: HALO C18 2.7 μm , 3.0 mm $\times$ 30 mm. Mobile Phase: ACN (0.05% TFA) - water (0.05% TFA). Gradient: MeCN from 5% to 95% over 1.4 min, hold 0.6 min, total run time is 2.5 min, flow rate: 1.8 mL/min. Column temperature: 50 °C. Wavelength: 214 and 254 nm PDA.
- Method 6: Equipment: Shimadzu LCMS 2020 Mass Spectrometer. Column: Kinetex C18 2.6 μm , 4.6 mm $\times$ 50 mm. Mobile Phase: ACN (0.05% TFA) - water (0.05% TFA); Gradient: MeCN from 5% to 95% over 1.8min, hold 0.7 min, total run time is 3.0 min, flow rate: 1.8 mL/min. Column temperature: 50 °C. Wavelength: 214 and 254 nm PDA.

10.3.2 Aggregates: Size Exclusion Chromatography (SEC)

The aggregate content of the conjugates was determined using the following method:

15	Equipment 1	UPLC Waters Acquity
	Equipment 2	UPLC Waters Acquity H-Class plus Bio
	Detector	Tunable UV detector (TUV)
	Detector cell	Titanium
	Pre-column	Agilent AdvanceBio SEC 300 Å 2.7 μm 4.6*50 mm
20	Column	Agilent AdvanceBio SEC 300 Å 2.7 μm 4.6*150 mm
	Mobile phase	Potassium Phosphate 50 mM pH 6.8 / 250 mM KCl
	Wavelength	280 nm
	Injection volume	10 μL
	Column temperature	Ambient
25	Sample manager temperature	25 °C
	Run time	10 min
	Flow	0.35 mL/min
	Isocratic mode	
	Weak wash	H ₂ O / ACN (90/10 v/v)
30	Strong wash	H ₂ O / ACN (10/90 v/v)

Mobile Phase preparation: Potassium phosphate 50mM pH 6.8 / 250mM KCl

- Weight 3.61 g KH₂PO₄ and 4.09 g K₂HPO₄ into a 1L volumetric flask. Complete with milliQ water. If necessary, adjust pH with HCl or NaOH 1 mol/L. Add 18.64 g of KCl.

Sample preparation: Prepare an ADC solution between 1 and 2.5 mg/mL in milliQ water.

10.3.3 DAR: Reverse Phase Liquid Chromatography (RPLC)

	Equipment	UPLC Waters Acquity
	Detector	Tunable UV detector (TUV)
5	Detector cell	Titanium
	Column	Waters Bioresolve 2.1*150 2.7 um Part No. 186008946
	Mobile phase A	Water + TFA 0.1% (v/v)
	Mobile phase B	ACN + TFA 0.1% (v/v)
	Wavelength	280 nm
10	Injection volume	10 µL
	Column temperature	90 °C
	Sample manager temperature	25 °C
	Run time	10 min
	Flow	0.35 mL/min
15	Weak wash	H ₂ O / ACN (90/10 v/v)
	Strong wash	H ₂ O / ACN (10/90 v/v)

- Gradient_basic:

t (min)	Flow (mL/min)	% A	% B
0.0	0.6	73	27 20
13.0	0.6	60	40
13.1	0.6	73	27
16.0	0.6	73	27

25

- Gradient dev2:

t (min)	Flow (mL/min)	% A	% B
0	0.6	73	27
16	0.6	50	50
16.1	0.6	40	60
17	0.6	40	60 30
17.1	0.6	73	27
19	0.6	73	27

35

- Gradient dev7:

t (min)	Flow (mL/min)	% A	% B
0	0.6	70	30
40	0.6	40	60
42	0.6	40	60
42.1	0.6	70	30 40
44	0.6	70	30

- Gradient dev7_short:

t (min)	Flow (mL/min)	% A	% B
0	0.6	70	30
20	0.6	55	45
20.1	0.6	5	95
23	0.6	5	95
23.1	0.6	70	30
25	0.6	70	30

- Mobile Phase preparation:

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ACN + 0.1 % TFA (V/V): Measure 1000 mL of ACN in a graduated cylinder and pour into a 1 L glass bottle. Add 1 mL of TFA with a 1 mL pipette and shake vigorously.

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WATER + 0.1% TFA (V/V): Measure 1000mL of ultrapure water in a graduated cylinder and pour into a 1L glass bottle. Add 1 mL of TFA with a 1 mL pipette and shake vigorously.

- Sample preparation:

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mAb: Prepare a 2.5 mg/mL mAb solution in milliQ water. In a vial dispense 45 μ L of this solution and 5 μ L of a 1 mol/L DTT solution. Incubate 30 minutes at 45 °C.

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ADC: Prepare a 2.5 mg/mL ADC solution in milliQ water. In a vial dispense 45 μ L of this solution and 5 μ L of a 0.1 mol/L DTT solution. Incubate 1 hour at 30 °C.

10.3.4 DAR: MS method

- Sample preparation:

Samples were diluted twice with 50 mM ammonium acetate and 25 μ g were injected for each sample.

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- UPLC:

The separation was performed using the MAbPac™ SEC-1 column (Thermo Scientific) and 50 mM ammonium acetate, pH 7, at 0.3 mL/min as mobile phase.

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- MS:

MS was performed using a QExactive HF Orbitrap operated in the high mass range. MS spectra were acquired in the 1800-8000 m/z at a resolution set to 15k, SID 50eV. The mass spectra were deconvoluted using Protein Deconvolution (Thermo Scientific).

10.3.5 Concentration: UV method

- Equipment:

UV spectrophotometer BioTek Synergy HT

5

- Buffer preparation:

PBS pH 7.4: Weight 8.0 g NaCl, 0.2 g KCl, 1.44 g Na₂HPO₄·2H₂O and 0.24 g KH₂PO₄ into a 1 L volumetric flask. Add 900 mL milliQ water. Mix it, when all salts are soluble, adjust pH between 7.35 and 7.44 with HCl 1 mol/L. Complete to 1 L with milliQ water.

10

- Sample preparation:

Prepare a 1 mg/mL ADC solution in PBS pH 7.4. Using a UV reader determine the absorbance at 252 and 280 nm.

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- Calculation formula:

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$$C^{mAb} = \left(\frac{A_{280} \times \epsilon_{252}^{DM1} - A_{252} \times \epsilon_{280}^{DM1}}{\epsilon_{280}^{mAb} \times \epsilon_{252}^{DM1} - \epsilon_{252}^{mAb} \times \epsilon_{280}^{DM1}} \right) / l$$

A= absorbance

DM1 (drug)= relevant payload

L = path length [cm]

25

C = concentration [mol/L]

ε = extinction coefficient [L.mol⁻¹.cm⁻¹]

c mg/mL = c mol/L * MW ADC

MW ADC = MW mAb + (MW payload * DAR moyen)

30

10.3.6 Purification

When prepared, the compounds were purified by Preparative Reverse Phase-HPLC on a Buchi C835 using a Waters column (XSelect CSH130 C18 5μm 19x150mm OBD or XBridge Prep C18 5μm OBD 19x150mm or XSELECT CSH Prep Fluoro-Phenyl 5μm 19x150mm) with the indicated solvent system at a flow rate of 25 mL/min. The elution was monitored by UV at a wavelength of 220 nm and by ELSD. Alternatively, the compounds were purified on Biotage Slegt System using Sfar C18 columns. The

35

purity of the peptides was determined with the UPLC methods described previously (see 9.3.1).

10.3.7 pH 8 DPBS and pH8 10x DPBS

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pH 8 DPBS and pH8 10x DPBS were obtained by addition of 1M NaOH to DPBS and 10x DPBS, respectively.

10.3.8 Naratuximab

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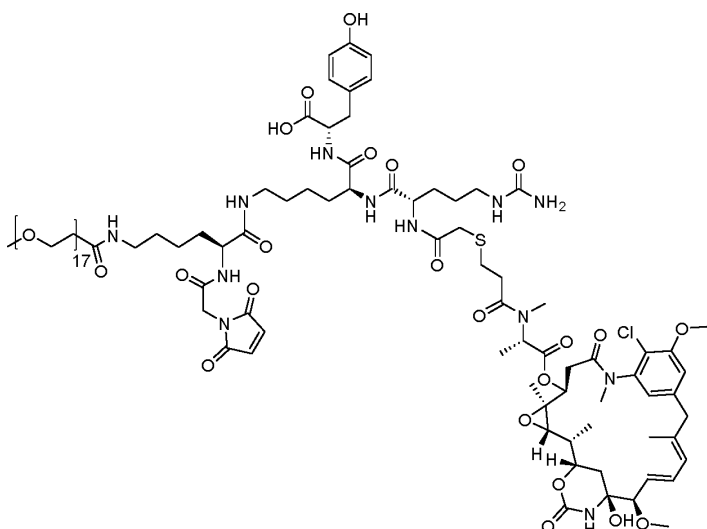
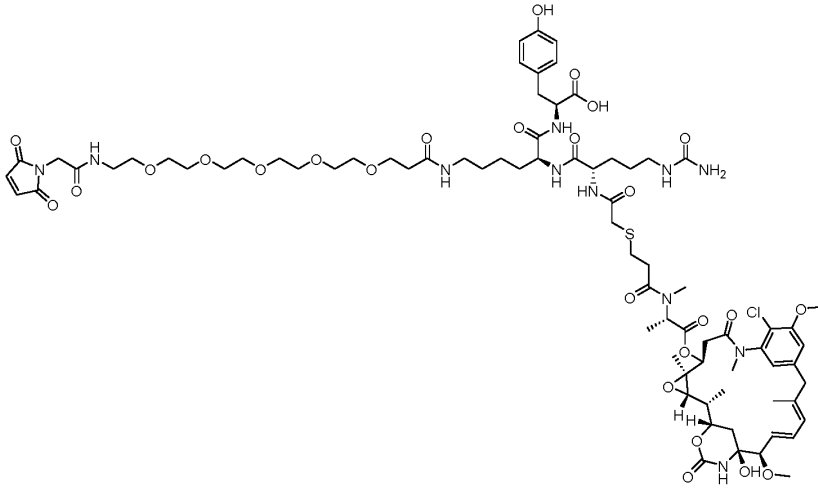
Naratuximab (also referred to as K7153A herein) refers to the antibody huCD37-3 (Version 1.0) described in WO 2019/229677 (incorporated herein by reference).

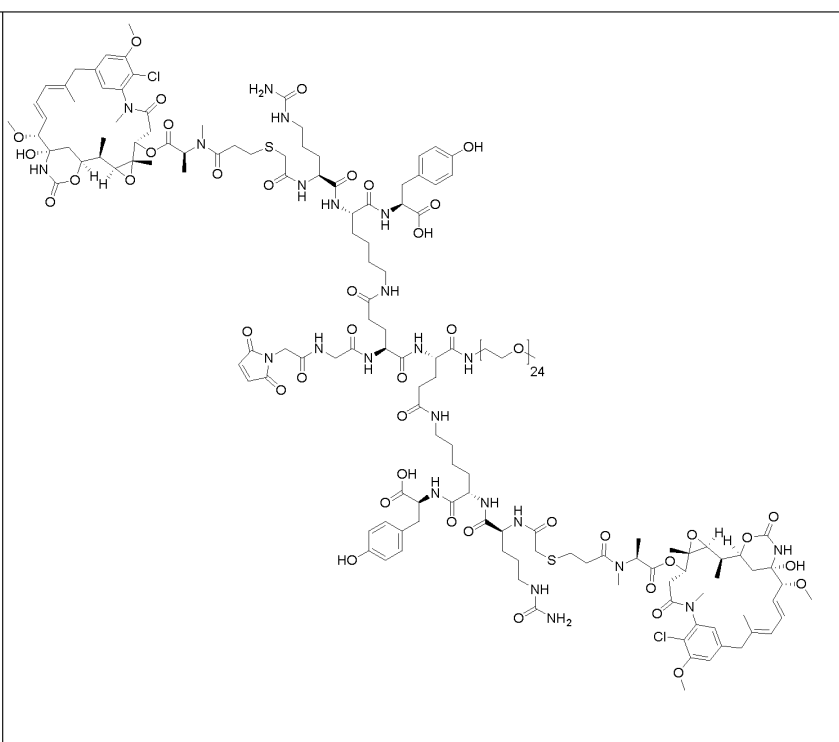
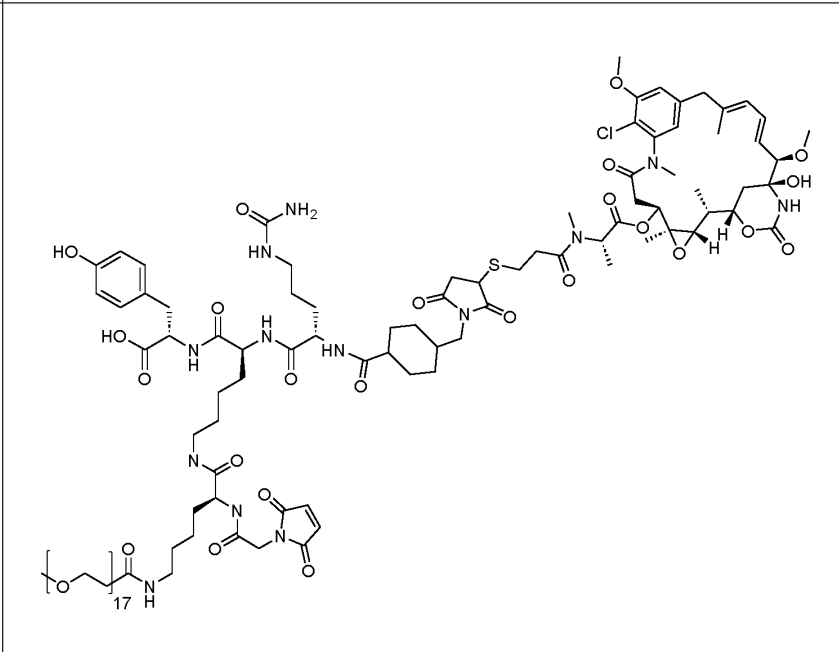
10.4 Preparation of Linker Payloads:

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The linker-payloads were prepared using standard chemistry methods and convergent strategies. The linker-payloads prepared in Example 10.4.1 to 10.4.5 and 10.4.7 are shown in Table 1 below.

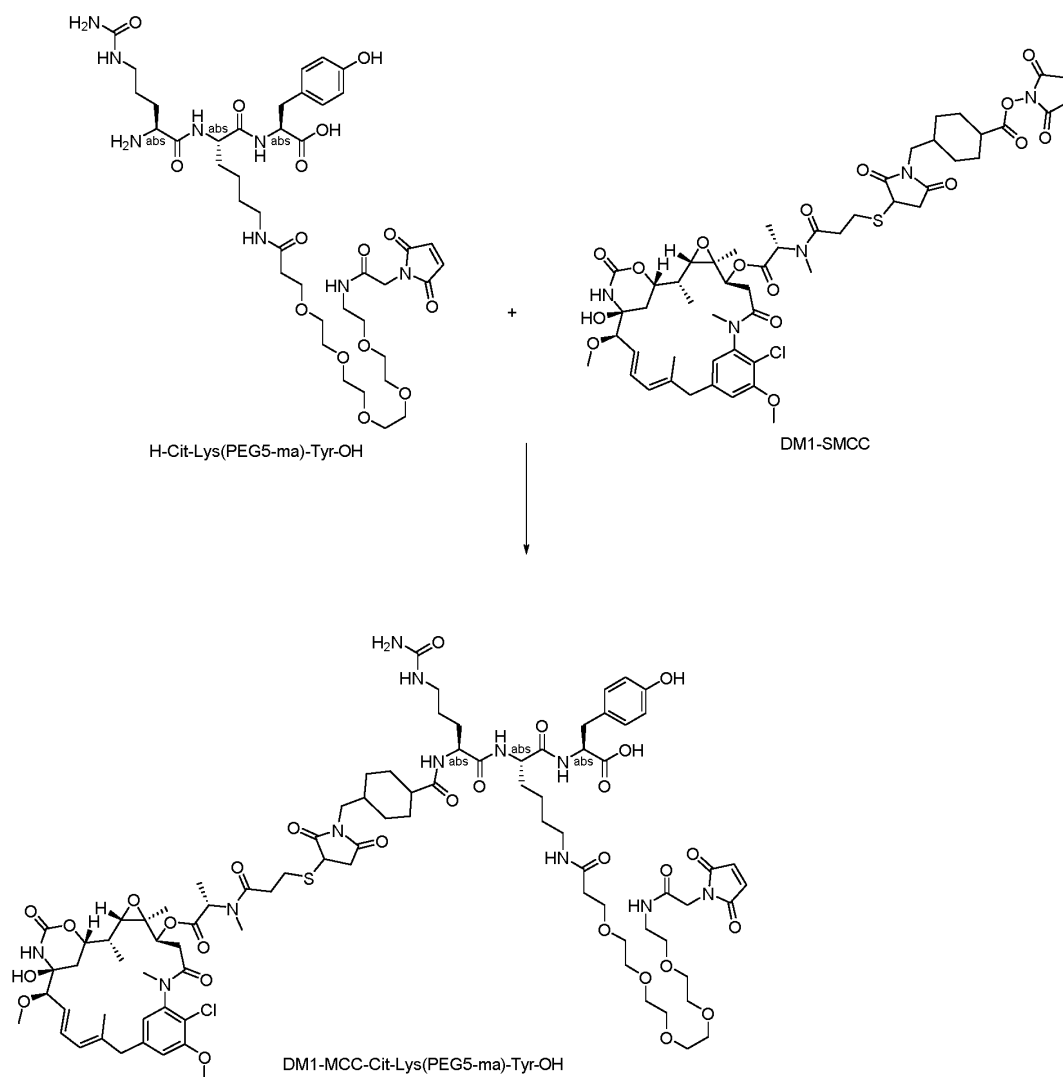
Linker-payload	Structure
DM1-MCC-Cit-Lys(ma-PEG5)-Tyr-OH	

DM1-Ac-Cit- Lys(ma- Lys(PEG16))- Tyr-OH	 The chemical structure shows a DM1 molecule (a complex macrocyclic cytotoxic agent) linked via a thioether bond to a lysine residue. This lysine is part of a peptide chain that includes a citrulline (Cit) residue and a lysine (Lys) residue. The Lys(ma-Lys(PEG16)) moiety is attached to the Cit residue. The PEG16 chain is represented by a long, flexible poly(ethylene glycol) segment. The Tyr-OH group is at the C-terminus of the peptide chain.
DM1-Ac-Cit- Lys(ma- PEG5)-Tyr-OH	 The chemical structure shows a DM1 molecule linked via a thioether bond to a lysine residue. This lysine is part of a peptide chain that includes a citrulline (Cit) residue and a lysine (Lys) residue. The Lys(ma-PEG5) moiety is attached to the Cit residue. The PEG5 chain is represented by a shorter, flexible poly(ethylene glycol) segment compared to the PEG16 in the first structure. The Tyr-OH group is at the C-terminus of the peptide chain.

ma-Gly- Glu(DM1-Ac- Cit-Lys-Tyr- OH)-Glu(DM1- Ac-Cit-Lys- Tyr-OH)- PEG24	
DM1-MCC-Cit- Lys(ma- Lys(PEG16))- Tyr-OH	

**DM1-Ac-Cit-
Lys(D-Arg-
ma)-Tyr-OH**

Table 1: linker-payloads

Example 10.4.1: Preparation of DM1-MCC-Cit-Lys(ma-PEG5)-Tyr-OH

H-Cit-Lys(PEG5-ma)-Tyr-OH was purchased from Ambipharm and prepared according to the following general procedure:

5

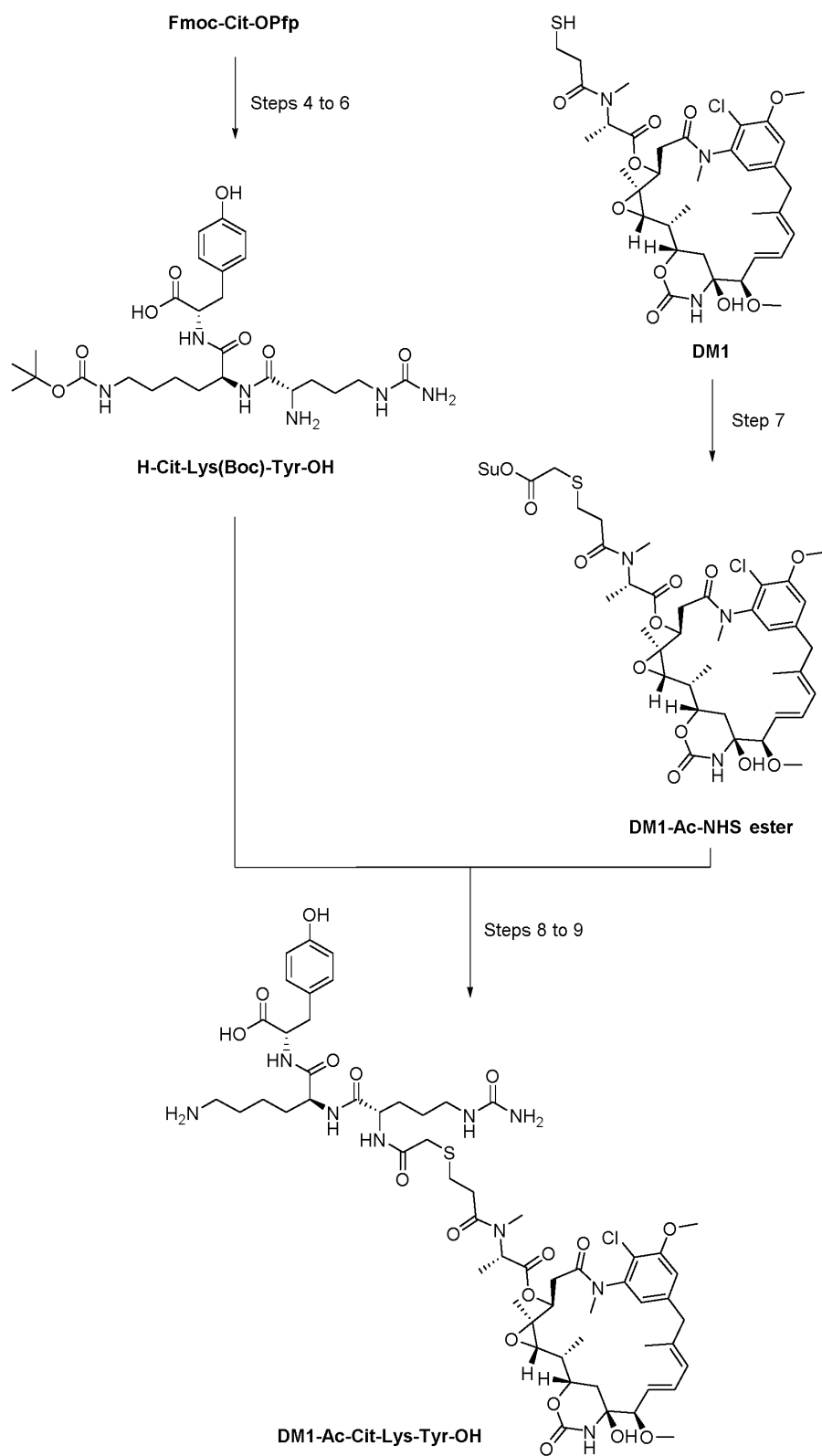
Peptide synthesis was performed on 2-CTC resin according to the general Fmoc/tBu strategy of solid phase peptide synthesis, with carboxyl group activation carried out by diisopropyl carbodiimide/HOBT. Sequentially, each amino acid was coupled as an active ester to the peptide chain, starting with the C-terminal amino acid. The final amino acid in the sequence was coupled with an N-terminally protected Boc group.

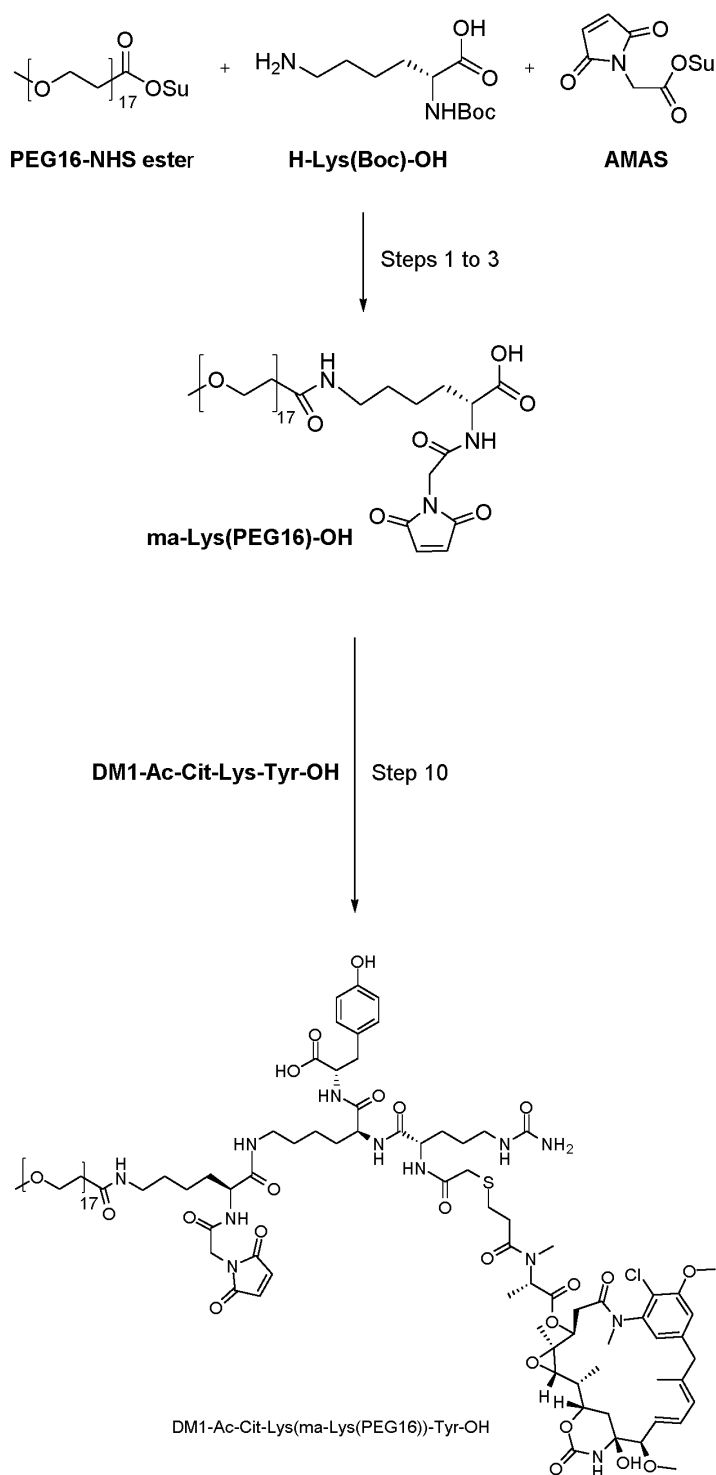
10 The Lys derivative was incorporated with the side chain amino group protected by ivDde which was removed with 2% hydrazine in DMF. Following ivDde removal, the

side chain was derivatized with maleimido-PEG₅-OH using the activated ester. Subsequently, the peptide was treated with a TFA-based acidolytic cocktail which resulted in its cleavage from the resin and deprotection of the side chain groups. The peptide was then purified by liquid chromatography (RP-HPLC). The purified peptide
5 TFA salt was lyophilized and obtained as a white to off-white powder.

DIEA (20 μ L, 0.11 mmol, 1.0 eq.) was added to a solution of H-Cit-Lys(PEG₅-ma)-Tyr-OH (100 mg, 0.11 mmol, 1.0 eq.) and DM1-SMCC (131.8 mg, 0.12 mmol, 1.1 eq.) in DMF (1 mL) at rt. After stirring at rt for 4 h, TFA was added until acidic pH was reached.
10 Purification by preparative HPLC (20 to 80% of ACN+0.1% TFA in water+0.1% TFA) afforded DM1-MCC-Cit-Lys(ma-PEG₅)-Tyr-OH (138.5 mg, 74.8 μ mol, 100% UV purity, 67% yield) as a white powder after freeze-drying. UPLC-MS (method 4): Rt = 2.58 and 2.63 min, m/z = 1854 [M+H]⁺, 1852 [M-H]⁻.

15 **Example 10.4.2: Preparation of DM1-Ac-Cit-Lys(ma-Lys(PEG₁₆))-Tyr-OH**





Step 1. DIEA (0.84 mL, 4.82 mmol, 5.0 eq.) was added to a mixture of mPEG16-NHS ester (900 mg, 0.96 mmol, 1.0 eq.) and Boc-Lys-OH (300 mg, 1.16 mmol, 1.2 eq.) in

DMF (12 mL) at rt. After stirring at rt for 16 h, TFA was added until acidic pH was reached. Purification by preparative HPLC (20 to 80% of ACN+0.1% TFA in water+0.1% TFA) afforded Boc-Lys(PEG16)-OH (990 mg, 0.95 mmol, 100% UV purity, 99% yield) as a colourless oil after freeze-drying. UPLC-MS (method 1): Rt = 1.16 min, m/z = 1038 [M+H]⁺.

Step 2. TFA (6 mL) was added to a solution of Boc-Lys(PEG16)-OH (990 mg, 0.95 mmol, 1.0 eq.) in DCM (6 mL) at rt. After stirring at rt for 30 min, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in a mixture of ACN/water (1:1, 16 mL) then freeze-dried to afford H-Lys(PEG16)-OH.TFA (1.00 g, 0.95 mmol, 100% UV purity, quant.) as a colourless oil. UPLC-MS (method 1): Rt = 0.89 min, m/z = 938 [M+H]⁺, 936 [M-H]⁻.

Step 3. DIEA (0.15 mL, 0.85 mmol, 4.0 eq.) was added to a mixture of H-Lys(PEG16)-OH (200 mg, 0.21 mmol, 1.0 eq.) and AMAS (56.5 mg, 0.22 mmol, 1.05 eq.) in DMF (2.5 mL) at rt. After stirring at rt for 1 h, TFA was added until acidic pH was reached. Purification by preparative HPLC (10 to 40% ACN+0.1% TFA in water+0.1% TFA) afforded ma-Lys(PEG16)-OH (220 mg, 0.20 mmol, 100% UV purity, 96% yield) as a colourless oil after freeze-drying. UPLC-MS (method 4): Rt = 1.74 min, m/z = 1075 [M+H]⁺, 1073 [M-H]⁻.

Step 4. DIEA (0.6 mL, 3.44 mmol, 3.7 eq.) was added to a mixture of Fmoc-Cit-OPFP (520.7 mg, 0.92 mmol, 1.0 eq.) and H-Lys(Boc)-OH (218.1 mg, 0.89 mmol, 0.96 eq.) in a mixture of DMF (6 mL) and water (3 mL) at rt. After stirring at rt for 1 h, TFA was added dropwise until acidic pH was reached. Purification by C18 flash column chromatography (20 to 80% of ACN+0.1% TFA in water+0.1% TFA) afforded Fmoc-Cit-Lys(Boc)-OH (593.3 mg, 0.92 mmol, 97% UV purity, quant.) as a white powder after freeze-drying. UPLC-MS (method 3): Rt = 1.80 min, m/z = 626 [M+H]⁺, 526 [M-Boc+H]⁺, 624 [M-H]⁻.

Step 5. H-Tyr-OMe (139.2 mg, 0.71 mmol, 1.1 eq.) followed by HATU (334.2 mg, 0.88 mmol, 1.4 eq.) and DIEA (0.5 mL, 2.87 mmol, 4.5 eq.) were added to a solution of Fmoc-Cit-Lys(Boc)-OH (400 mg, 0.64 mmol, 1.0 eq.) in DMF (10 mL) at rt. After stirring at rt for 2 h, TFA was added until acidic pH was reached. Purification by C18 flash column chromatography (20 to 80% ACN+0.1% TFA in water+0.1% TFA) gave H-Cit-Lys(Boc)-Tyr-H (513.3 mg, 0.57 mmol, 89% UV purity, 89% yield) as a white powder after freeze-drying. UPLC-MS (method 3): Rt = 1.83 min, m/z = 803 [M+H]⁺, 703 [M-Boc+H]⁺, 847 [M+FA-H]⁻.

Step 6. Lithium hydroxide monohydrate (158.5 mg, 3.78 mmol, 4.2 eq.) was added to a solution of Fmoc-Cit-Lys(Boc)-Tyr-OMe (730.6 mg, 0.91 mmol, 1.0 eq.) in THF (8 mL) and water (5 mL) at rt. After stirring at rt for 40 min, TFA was added until slightly acidic pH was reached then the reaction mixture was concentrated *in vacuo*. Purification by preparative HPLC (15 to 40% of ACN+0.1% TFA in water+0.1% TFA) afforded H-Cit-Lys(Boc)-Tyr-OH (510 mg, 0.90 mmol, 100% UV purity, 99% yield) as a white powder after freeze-drying. UPLC-MS (method 1): Rt = 0.80 min, m/z = 567 [M+H]⁺, 565 [M-H]⁻.

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Step 7. DIEA (0.28 mL, 1.63 mmol, 6.0 eq.) was added to a solution of bromoacetic acid (64.8 mg, 0.47 mmol, 1.7 eq.) and DM1 (200 mg, 0.27 mmol, 1.0 eq.) in DMF (2 mL) at rt. After stirring at rt for 1 h, a mixture of HATU (113.3 mg, 0.30 mmol, 1.1 eq.) and 1-hydroxypyrrolidine-2,5-dione (34.3 mg, 0.30 mmol, 1.1 eq.) was added. After 30 min stirring at rt, TFA was added until acidic pH was reached. Purification on preparative HPLC (30 to 60% ACN+0.1% TFA in water+0.1% TFA) afforded DM1-Ac-NHS ester (134.7 mg, 0.12 mmol, 80% UV purity, 45% yield) as a white powder after freeze-drying. UPLC-MS (method 2): Rt = 1.57 min, m/z = 891 [M-H]⁻.

Step 8. DIEA (0.22 mL, 1.24 mmol, 4.0 eq.) was added to a mixture of DM1-Ac-NHS ester (347 mg, 0.31 mmol, 1.0 eq.) and H-Cit-Lys(Boc)-Tyr-OH (222.1 mg, 0.33 mmol, 1.05 eq.) in DMF (4 mL) at rt. After stirring at rt for 1 h, TFA was added until acidic pH was reached. Purification by preparative HPLC (30 to 60% of ACN+0.1% TFA in water+0.1% TFA) afforded DM1-Ac-Cit-Lys(Boc)-Tyr-OH (417 mg, 0.31 mmol, 100% UV purity, quant.) as a white powder after freeze-drying. UPLC-MS (method 3): Rt = 1.67 min, m/z = 1344 [M-H]⁻.

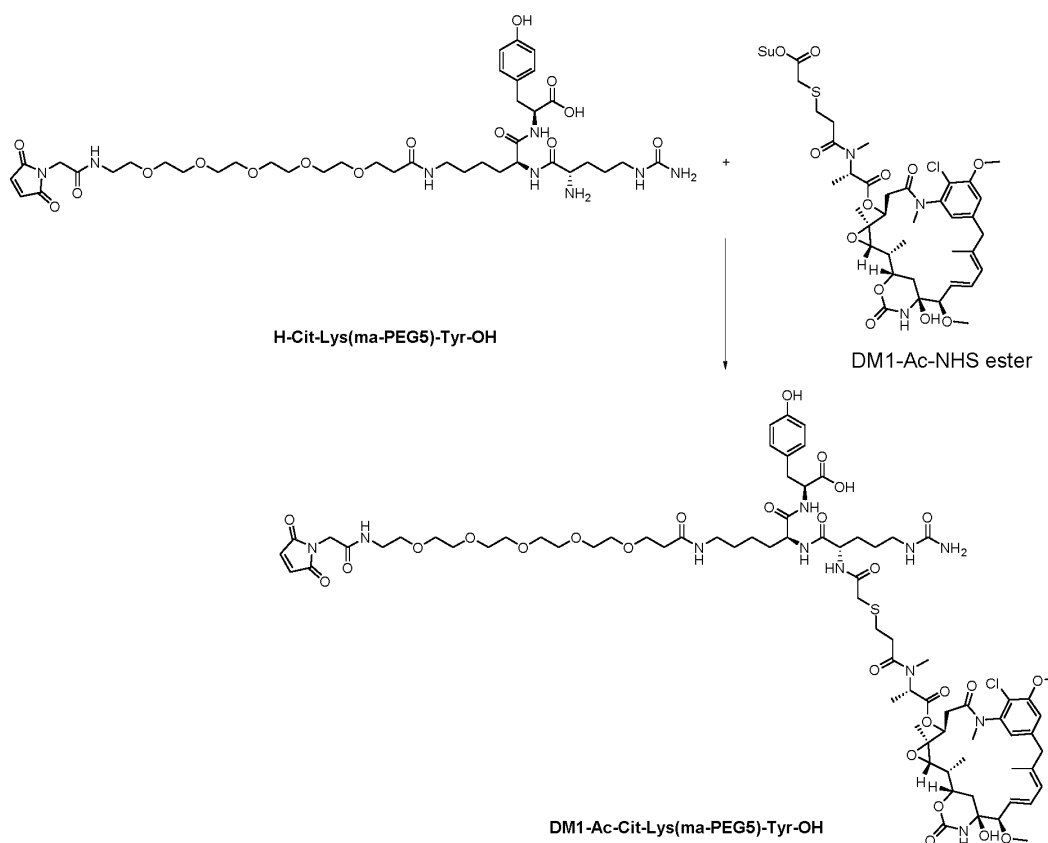
Step 9. A mixture of TFA (0.9 mL) and DCM (3.6 mL) was added to a mixture of DM1-Ac-Cit-Lys(Boc)-Tyr-OH (112.0 mg, 83.3 μmol, 1.0 eq.) at rt. After stirring at rt for 5 min, a mixture of ACN/water (1:1, 8 mL) was added to the reaction mixture. The DCM was removed by concentration under vacuo. Purification by preparative HPLC (2 to 40% of ACN+0.1% TFA in water+0.1% TFA) afforded DM1-Ac-Cit-Lys-Tyr-OH (80 mg, 64.3 μmol, 100% UV purity, 77% yield) as a white powder after freeze-drying. UPLC-MS (method 2): Rt = 1.03 min, m/z = 1242 [M+H]⁺, 1240 [M-H]⁻.

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Step 10. 1-hydroxypyrrolidine-2,5-dione (2.14 mg, 18.6 μmol, 1.0 eq.) and EDC.HCl (3.57 mg, 18.6 μmol, 1.0 eq.) were added to a solution of ma-Lys(PEG16)-OH (20.0 mg, 18.6 μmol, 1.0 eq.) in DCM (2 mL) at rt. After stirring at rt for 2.5 h, DM1-Ac-Cit-

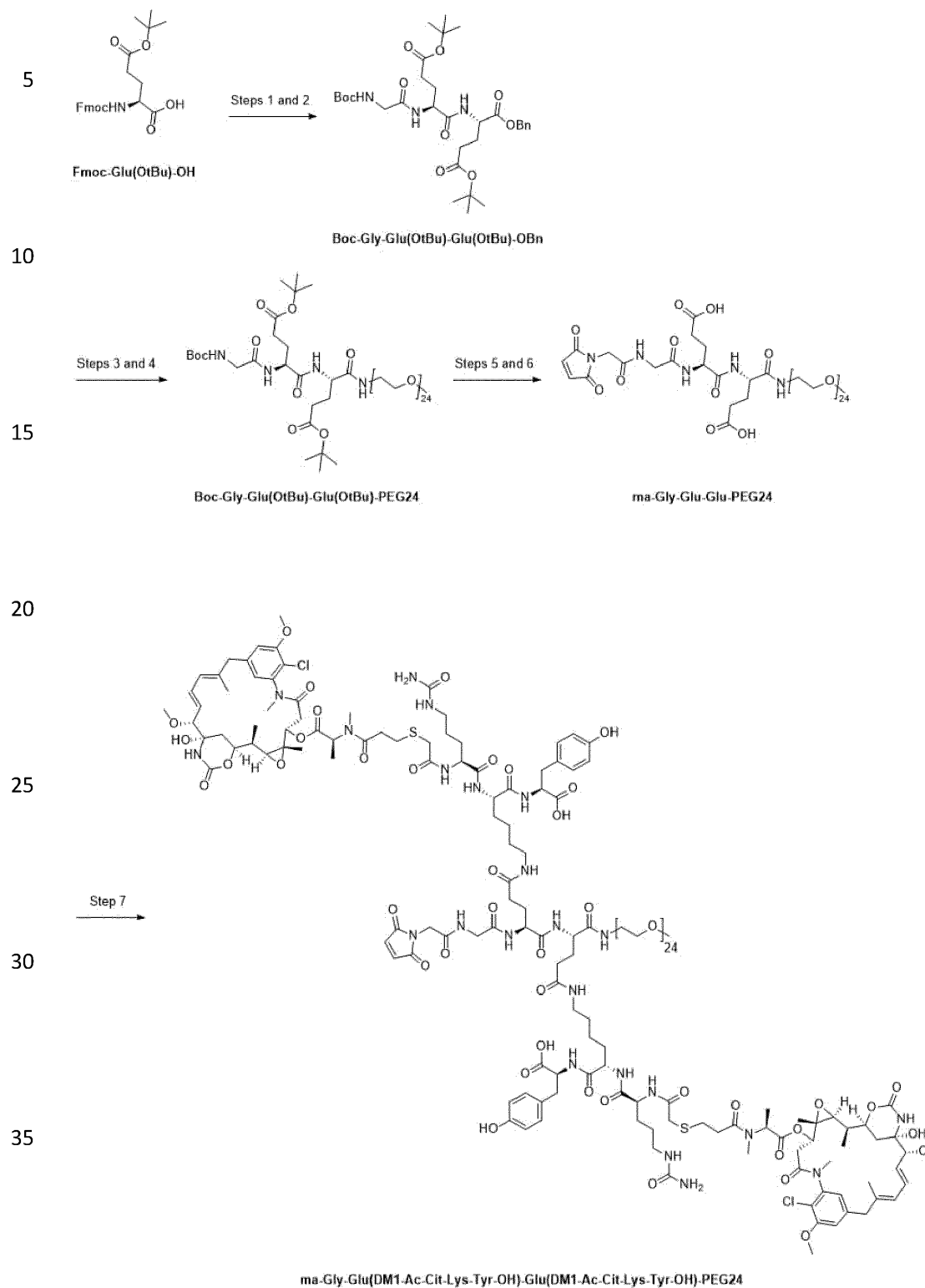
Lys-Tyr-OH (37.1 mg, 22.3 μmol , 1.2 eq.) and DIEA (13 μL , 74.5 μmol , 4.0 eq.) were added to the reaction mixture at rt. After stirring at rt for 10 min, TFA was added until acidic pH was reached. Purification by preparative HPLC (5 to 100% of ACN+0.1% FA in water+0.1% FA) afforded DM1-Ac-Cit-Lys(ma-Lys(PEG16))-Tyr-OH (17.2 mg, 7.50 μmol , 100% UV purity, 40% yield) as a white powder after freeze drying. UPLC-MS (method 4): R_t = 2.59 min, m/z = 1173 $[\text{M}+\text{FA}-\text{H}]^-$.

Example 10.4.3: Preparation of DM1-Ac-Cit-Lys(ma-PEG5)-Tyr-OH



DIEA (31 μL , 0.18 mmol, 4.0 eq.) was added to a mixture of DM1-Ac-NHS ester (50.0 mg, 44.8 μmol , 1.0 eq.) and H-Cit-Lys(ma-PEG5)-Tyr-OH. TFA (45.2 mg, 44.8 μmol , 1.0 eq.) in DMF (1 mL) at rt. After stirring at rt for 30 min, TFA was added until acidic pH was reached. Purification by preparative HPLC (20 to 50% of ACN+0.1%TFA in water+0.1%TFA) afforded DM1-Ac-Cit-Lys(ma-PEG5)-Tyr-OH (74.9 mg, 44.8 μmol , 100% UV purity, quant.) as a white powder after freeze-drying. UPLC-MS (method 1): R_t = 1.31 min, m/z = 1672 $[\text{M}-\text{H}]^-$.

Example 10.4.4: Preparation of ma-Gly-Glu(DM1-Ac-Cit-Lys-Tyr-OH)-Glu(DM1-Ac-Cit-Lys-Tyr-OH)-PEG24



Step 1. DIEA (1.17 mL, 6.66 mmol, 3.0 eq.) was added to a mixture of Fmoc-Glu(OtBu)-OH.H₂O (1.00 g, 2.22 mmol, 1.0 eq.), H-Glu(OtBu)-OBn.HCl (750 mg, 2.22 mmol, 1.0 eq.) and HATU (1.14 g, 2.99 mmol, 1.35 eq.) in DMF (15 mL) at 0 °C. After stirring at 0 °C for 15 min and at rt for 1 h, the reaction mixture was concentrated to dryness. The resulting brown oil was diluted with EtOAc and washed with a 5% aqueous NaHCO₃ solution. The organic phase was washed with brine, dried over MgSO₄, filtered and concentrated to dryness. Flash chromatography (*n*-heptane:EtOAc 80:20 to 0:100) afforded Fmoc-Glu(OtBu)-Glu(OtBu)-OBn (1.46 g, 2.09 mmol, 100% UV purity, 94% yield) as a white foam. UPLC-MS (method 3): Rt = 2.62 min, m/z = 701 [M+H]⁺, 745 [M+FA-H]⁻.

Step 2. DBU (0.3 mL, 2.00 mmol, 1.2 eq.) was added to a solution of Fmoc-Glu(OtBu)-Glu(OtBu)-OBn (1.17 g, 1.67 mmol, 1.0 eq.) in DMF (5mL) at rt. After stirring at rt for 5 min, the reaction mixture was added dropwise over 3 min to a mixture of Boc-Gly-OH (351 mg, 2.00 mmol, 1.2 eq.), HATU (855.3 mg, 2.17 mmol, 1.3 eq.) and DIEA (0.87 mL, 5.01 mmol, 3.0 eq.) in DMF (12 mL) at rt. After stirring at rt for 30 min, the reaction mixture was concentrated to dryness. The residue was diluted with EtOAc then washed with citric acid 10%. The organic layer was dried over MgSO₄ and concentrated to dryness. Flash chromatography (DCM:EtOAc 90:10 to 0:100) afforded Boc-Gly-Glu(OtBu)-Glu(OtBu)-OBn (982 mg, 1.47 mmol, 95% UV purity, 88% yield) as a white solid. UPLC-MS (method 3): Rt = 2.22 min, m/z = 636 [M+H]⁺, 634 [M-H]⁻.

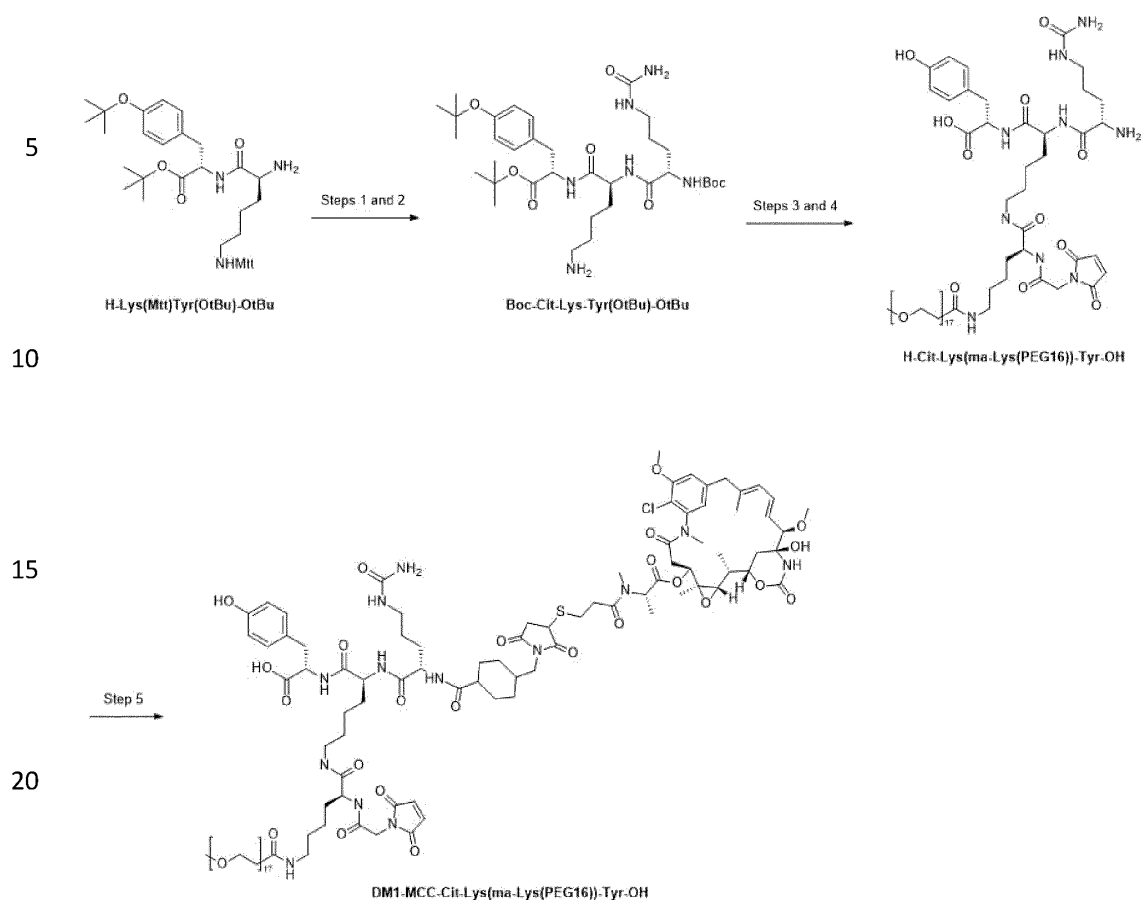
Step 3. Palladium (164.4 mg, 0.15 mmol, 0.1 eq.) was added under nitrogen atmosphere into a 25 mL flask for hydrogenation. A solution of Boc-Gly-Glu(OtBu)-Glu(OtBu)-OBn (982 mg, 1.54 mmol, 1.0 eq.) in ethanol (9.8 mL) was added. The resulting suspension was stirred under 1 bar of hydrogen at rt for 30 min then filtered through a pad of Celite®. The filtrate was concentrated under reduced pressure to afford Boc-Gly-Glu(OtBu)-Glu(OtBu)-OH (898 mg, 1.65 mmol, 94% UV purity, quant.) as a white foam. UPLC-MS (method 3): Rt = 1.73 min, m/z = 546 [M+H]⁺, 544 [M-H]⁻.

Step 4. DIEA (48 mL, 0.27 mmol, 3.0 eq.) was added to a mixture of Boc-Gly-Glu(OtBu)-Glu(OtBu)-OH (50.0 mg, 91.6 mmol, 1.0 eq.), PEG24-amine (120.5 mg, 0.11 mmol, 1.2 eq.) and HATU (59.0 mg, 0.15 mmol, 1.6 eq.) in DMF (0.5 mL) at rt. After stirring at rt for 10 min, the reaction mixture was acidified with TFA to pH 4-5. Purification by preparative HPLC (10-100% of ACN+0.1% TFA in water+0.1% TFA) afforded Boc-Gly-Glu(OtBu)-Glu(OtBu)-PEG24 (100 mg, 61.9 mmol, 100% UV purity, 68% yield) as a white solid after freeze-drying. UPLC-MS (method 3): Rt = 1.82 min, m/z = 1614 [M-H]⁻, 1660 [M+FA-H]⁻.

Step 5. TFA (0.5 mL) was added to a solution of Boc-Gly-Glu(OtBu)-Glu(OtBu)-PEG24 (100 mg, 61.9 mmol, 1.0 eq.) in DCM (0.5 mL) at rt. After stirring at rt for 30 min, the reaction mixture was concentrated to dryness to afford H-Gly-Glu-Glu-PEG24.TFA (93 mg, 61.3 mmol, 99% yield) as a yellow oil. UPLC-MS (method 1): Rt = 0.94 min, m/z = 1404 [M+H]⁺, 1402 [M-H]⁻.

Step 6. DIEA (53 µL, 0.31 mmol, 5.0 eq.) was added to a mixture of H-Gly-Glu-Glu-PEG24.TFA (93.0 mg, 61.3 µmol, 1.0 eq.) and AMAS (21.2 mg, 79.7 µmol, 1.3 eq.) in DMF (0.3 mL) at rt. After stirring at rt for 1 h, TFA was added (24 µL). Purification by preparative HPLC (20-100% of ACN+0.1% TFA in water+0.1% TFA) afforded ma-Gly-Glu-Glu-PEG24 (94.0 mg, 58.0 mmol, 95% UV purity, 95% yield) as colourless oil. UPLC-MS (method 3): Rt = 1.20 min, m/z = 1539 [M-H]⁻.

Step 7. DIEA (27 µL, 0.16 mmol, 8.0 eq.) was added to a mixture of ma-Gly-Glu-Glu-PEG24 (30.0 mg, 19.5 µmol, 1.0 eq.) and HATU (14.8 mg, 38.9 µmol, 2.0 eq.) in DMF (3 mL) at rt. After stirring at rt for 7 min, the reaction mixture was added to DM1-Ac-Cit-Lys-Tyr-OH (52.9 mg, 38.9 µmol, 2.0 eq.) at rt. After stirring at rt for 15 min, TFA was added until acidic pH was reached. Purification by preparative HPLC (30 to 70% of ACN+0.1% TFA in water+0.1% TFA) afforded ma-Gly-Glu(DM1-Ac-Cit-Lys-Tyr-OH)-Glu(DM1-Ac-Cit-Lys-Tyr-OH)-PEG24 (15.1 mg, 3.40 µmol, 89% UV purity, 17% yield) as a white powder after freeze-drying. UPLC-MS (method 3): Rt = 1.70 min, m/z = 1996 [M-2H]²⁻.

Example 10.4.5: Preparation of DM1-MCC-Cit-Lys(ma-Lys(PEG16))-Tyr-OH

Step 1. DIEA (0.7 mL, 3.98 mmol, 3.0 eq.) was added to a mixture of H-Lys(Mtt)-Tyr(OtBu)-OtBu (1.00 g, 1.33 mmol, 1.0 eq.), HATU (680 mg, 1.73 mmol, 1.3 eq.) and Boc-Cit-OH (373 mg, 1.33 mmol, 1.0 eq.) in DMF (8 mL) at 0-5 °C. After stirring at rt for 30 min, the reaction mixture was poured into EtOAc (200 mL), washed with brine (100 mL) then half saturated brine (50 mL). The resulting emulsion was first evaporated under reduced pressure then freeze-dried to afford Boc-Cit-Lys(Mtt)-Tyr(OtBu)-OtBu (2.10 g, 1.80 mmol, 60% purity, quant.) as a beige solid. UPLC-MS (method 3): Rt = 2.02 min, m/z = 936 [M+H]⁺, 980 [M+FA-H]⁻.

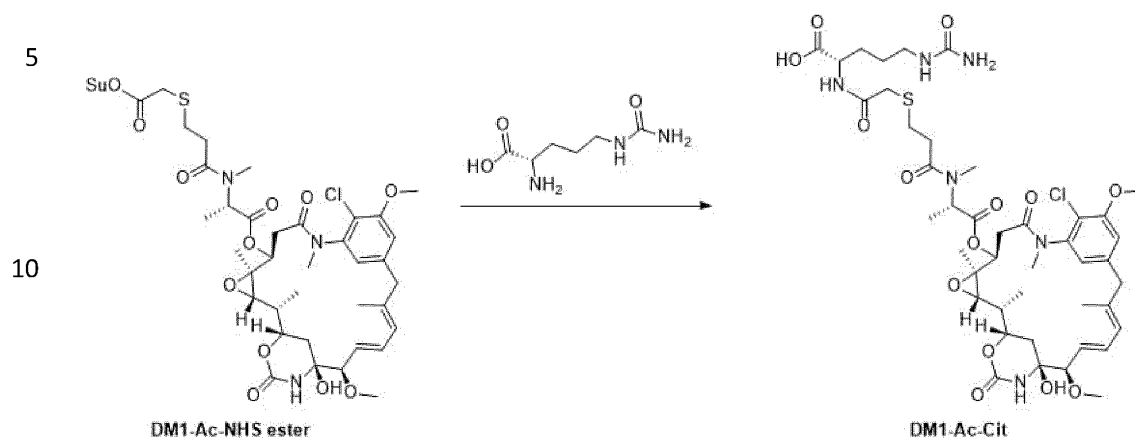
Step 2. A mixture of DCM (65 mL) and TFA (2 mL) was added to Boc-Cit-Lys(Mtt)-Tyr(OtBu)-OtBu (1.24 g, 1.33 mmol, 1.0 eq.) at rt. After stirring at rt for 10 min, the reaction mixture was partially concentrated then added slowly into cold ether. The oily product obtained by centrifugation was purified by preparative HPLC (25 to 80% of ACN+0.1% TFA in water+0.1% TFA) to afford Boc-Cit-Lys-Tyr(OtBu)-OtBu

(602 mg, 0.76 mmol, 100% UV purity, 57% yield) as a white powder after freeze-drying. UPLC-MS (method 3): Rt = 1.46 min, m/z = 680 [M+H]⁺, 723 [M+FA-H]⁻.

Step 3. DIEA (0.06 mL, 0.37 mmol, 4.0 eq.) was added to a mixture of ma-Lys(PEG16)-OH (100 mg, 93.1 μmol, 1.0 eq.), Boc-Cit-Lys-Tyr(OtBu)-OtBu (81.2 mg, 102.4 μmol, 1.1 eq.) and HATU (47.7 mg, 121 μmol, 1.3 eq.) in DMF (1.5 mL) at 5 °C. After stirring at 5 °C for 10 min, TFA was added until acidic pH was reached. Purification by preparative HPLC (20 to 70% of ACN+0.1% TFA in water+0.1% TFA) afforded Boc-Cit-Lys(ma-Lys(PEG16))-Tyr(OtBu)-OtBu (158 mg, mmol, 96% UV purity, 94% yield) as a white powder after freeze-drying. UPLC-MS (method 3): Rt = 1.81 min, m/z = 1736 [M+H]⁺, 1780 [M+FA-H]⁻.

Step 4. A mixture of TFA (2.5 mL) and DCM (2.5 mL) was added to Boc-Cit-Lys(ma-Lys(PEG16))-Tyr(OtBu)-OtBu (158 mg, 87.2 μmol, 1.0 eq.) at rt. After stirring at 4 °C for 16 h, the reaction mixture was concentrated in vacuo. Purification by preparative HPLC (10 to 50% of ACN+0.1% TFA in water+0.1% TFA) afforded H-Cit-Lys(ma-Lys(PEG16))-Tyr-OH (132 mg, 79.6 μmol, 99% UV purity, 91% yield) as a colourless oil after freeze-drying. UPLC-MS (method 3): Rt = 1.04 min, m/z = 1523 [M+H]⁺, 1521 [M-H]⁻.

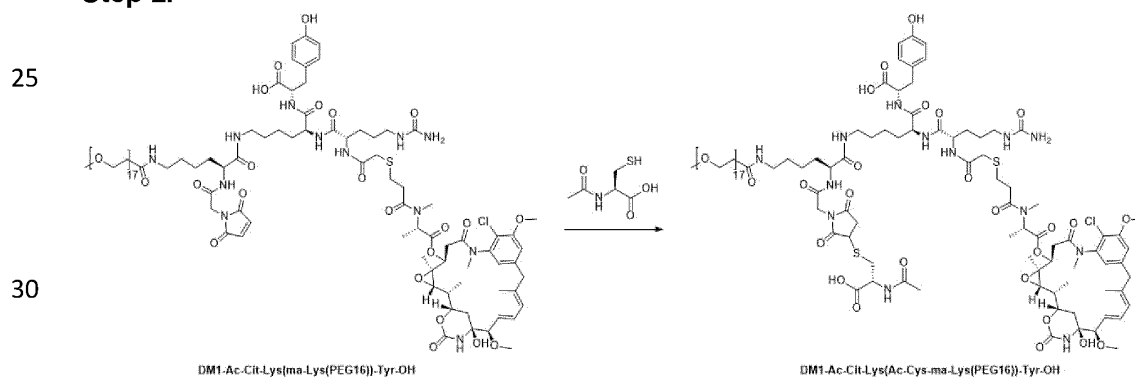
Step 5. DIEA (41 μL, 0.25 mmol, 4.0 eq.) was added to a mixture of DM1-SMCC (68.6 mg, 62.0 μmol, 1.0 eq.) and H-Cit-Lys(ma-Lys(PEG16))-Tyr-OH (103 mg, 62.0 μmol, 1.0 eq.) in DMF (1.5 mL) at rt. After stirring at rt for 1.5 h, TFA was added until acidic pH was reached. Purification by preparative HPLC (20 to 90% of ACN+0.1% TFA in water+0.1% TFA) afforded DM1-MCC-Cit-Lys(ma-Lys(PEG16))-Tyr-OH (73.4 mg, 29.4 μmol, 99% UV purity, 47% yield) as a white powder after freeze-drying. UPLC-MS (method 3): Rt = 1.60 and 1.62 min, m/z = 827 [M+3H]³⁺, 870 [M+FA-3H]³⁻.

Example 10.4.6: Preparation of DM1-Ac-Cit-Lys(Ac-Cys-ma-Lys(PEG16))-Tyr-OH**Step 1.**

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DIEA (0.14 mL, 0.83 mmol, 4.0 eq.) was added to a mixture of DM1-Ac-NHS ester (185 mg, 0.21 mmol, 1.0 eq.) and Citrulline (72.6 mg, 0.41 mmol, 2.0 eq.) in DMSO (3.7 mL) at rt. After stirring at rt for 18 h, TFA was added until acidic pH was reached. Purification by preparative HPLC (10 to 40% of can+0.1% TFA in water+0.1% TFA) afforded DM1-Ac-Cit (130 mg, 0.14 mmol, 100% UV purity, 66% yield) as a white powder after freeze-drying. UPLC-MS (method 1): Rt = 1.33 min, m/z = 952 [M-H]⁻.

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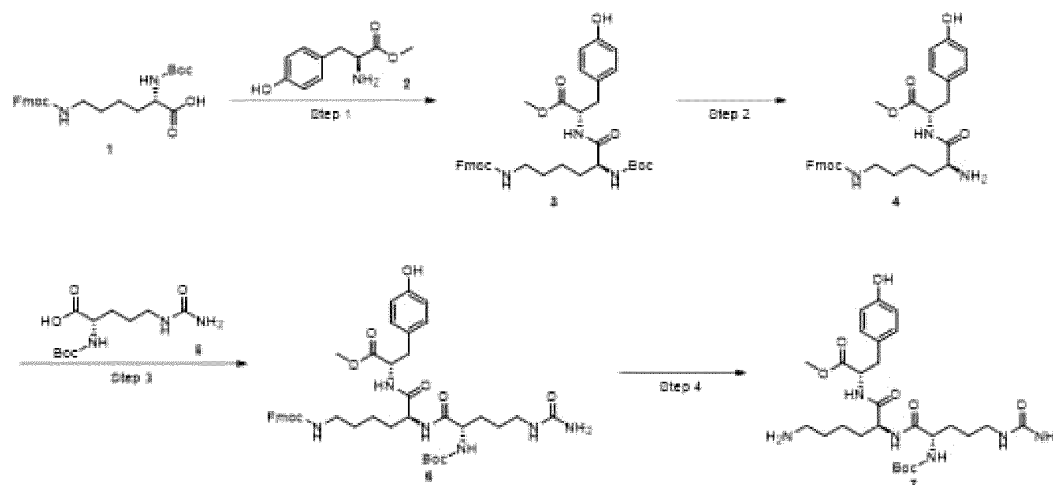
Step 2.

Acetyl-L-cysteine (0.32 mg, 2.17 μ mol, 1.0 eq.) was added to a solution of DM1-Ac-Cit-Lys(ma-Lys(PEG16))-Tyr-OH (5.0 mg, 2.17 μ mol, 1.0 eq.) in DMF (1 mL) at rt. After stirring at rt for 40 min, TFA was added until acidic pH was reached. Purification by preparative HPLC (5 to 100% can+0.1% TFA in water+0.1% TFA) afforded DM1-Ac-Cit-Lys(Ac-Cys-ma-Lys(PEG16))-Tyr-OH (4.59 mg, 1.86 μ mol, 99% UV purity, 94% yield)

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as a white powder after freeze-drying. UPLC-MS (method 4): $R_t = 2.41$ min, $m/z = 1231$ $[M-2H]^{2-}$.

5 Example 10.4.7: Preparation of DM1-Ac-Cit-Lys(D-Arg-ma)-Tyr-OH



Step 1. DIEA (22.0 g, 0.17 mol, 4.0 eq) was added to a mixture of Boc-Lys(Fmoc)-OH (20.0 g, 43.0 mmol, 1.0 eq), H-Tyr-OMe (8.30 g, 43.0 mmol, 1.0 eq) and HATU (21.0 g, 55.0 mmol, 1.3 eq) in DMF (150 mL) at rt. After stirring at rt for 16 h, the reaction mixture was poured into water (400 mL) and extracted with EtOAc (3 x 80 mL). The combined organic phases were dried over sodium sulfate, concentrated and purified by silica gel column chromatography (eluting with MeOH/DCM, 0% to 5%) to afford Boc-Lys(Fmoc)-Tyr-OMe (26.0 g, 32.6 mmol, 85% UV purity, 76% yield) as a white solid. LC-MS (method 5): $R_t = 0.71$ min, $m/z = 668.3$ $[M+Na]^+$.

Step 2. TFA (0.23 g, 2.00 mmol, 2.0 eq) was added to a solution of Boc-Lys(Fmoc)-Tyr-OMe (1.00 g, 1.00 mmol, 1.0 eq) in DCM (10 mL) at 0 °C. After stirring at rt for 1 h, the reaction mixture was concentrated to give H-Lys(Fmoc)-Tyr-OMe (800 mg, 0.70 mmol, 85% UV purity, 70% yield) which was used directly in next step without further purification. LC-MS (method 5): $R_t = 1.11$ min, $m/z = 546.3$ $[M+H]^+$.

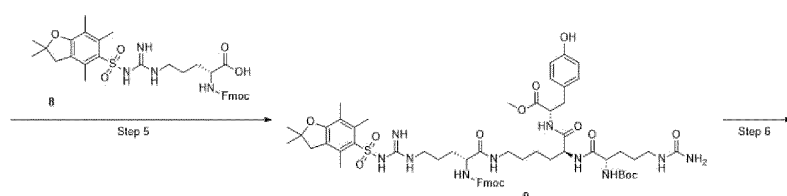
Step 3. DIEA (11.3 g, 87.8 mmol, 2.4 eq) was added to a mixture of H-Lys(Fmoc)-Tyr-OMe (20.0 g, 36.6 mmol, 1.0 eq), Boc-Cit-OH (10.1 g, 36.6 mmol, 1.0 eq) and HATU (15.3 g, 40.2 mmol, 1.1 eq) in DMF (100 mL) at rt. After stirring at rt for 16 h, the reaction mixture was poured into water (400 mL) and extracted with EtOAc (3 x 80 mL). The

combined organic phases were dried over sodium sulfate, concentrated and purified by silica gel column chromatography (eluting with MeOH/DCM, 0% to 5%) to afford Boc-Cit-Lys(Fmoc)-Tyr-OMe (12.6 g, 15.4 mmol, 90% UV purity, 42% yield) as a white solid. LC-MS (method 5): $R_t = 1.25$ min, $m/z = 803.4$ $[M+H]^+$.

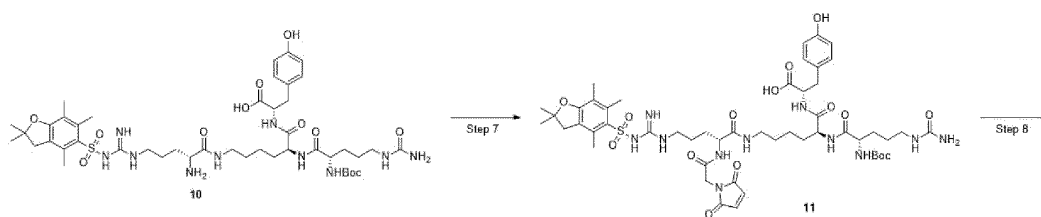
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Step 4. Diethylamine (1.36 g, 18.6 mmol, 3.0 eq) was added to a solution of Boc-Cit-Lys(Fmoc)-Tyr-OMe (5.00 g, 6.20 mmol, 1.0 eq) in DCM (10 mL) at rt. After stirring at rt for 10 h, the reaction mixture was concentrated, washed with Et₂O and triturated in Et₂O to afford Boc-Cit-Lys-Tyr-OMe (3.00 g, 4.59 mmol, 90% UV purity, 74% yield) which was used directly in next step without further purification. LC-MS (method 5): $R_t = 1.26$ min, $m/z = 581.4$ $[M+H]^+$.

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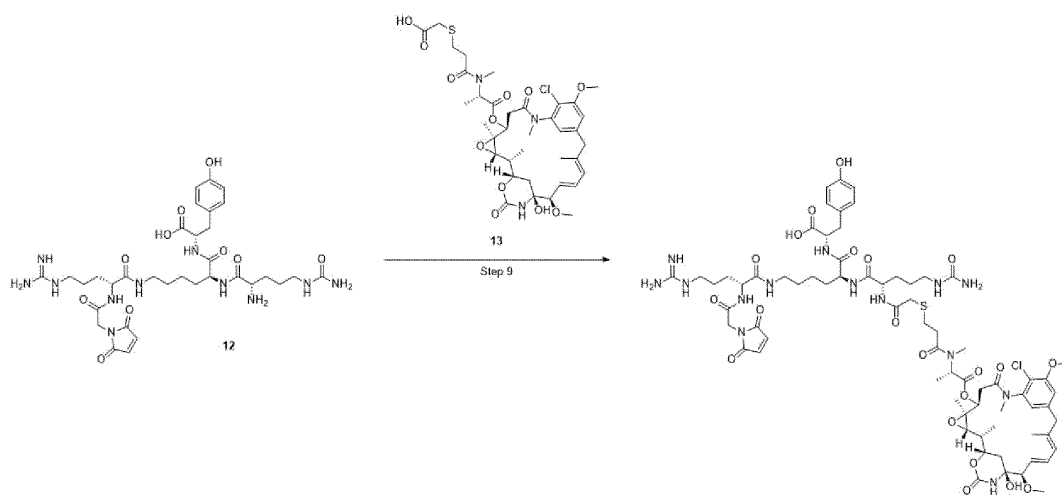


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DM1-Ac-Cit-Lys(D-Arg-ma)-Tyr-OH

Step 5. DIEA (0.80 g, 6.20 mmol, 2.0 eq) was added to a mixture of Boc-Cit-Lys-Tyr-OMe (1.98 g, 3.40 mmol, 1.1 eq), Fmoc-D-Arg(Pbf)-OH (2.00 g, 3.10 mmol, 1.0 eq) and HATU (1.30 g, 3.40 mmol, 1.1 eq) in DMF (30 mL) at rt. After stirring at rt for 16 h, the reaction mixture was poured into water (300 mL) and extracted with EtOAc (3 x 80 mL). The combined organic phases were dried over sodium sulfate, concentrated, and purified by silica gel column chromatography (eluting with MeOH/DCM, 0% to 5%) to afford Boc-Cit-Lys(Fmoc-D-Arg(Pbf))-Tyr-OMe (2.10 g, 2.26 mmol, 95% UV purity, 73% yield) as a white solid. LC-MS (method 6): Rt = 2.08 min, m/z = 1211.7 [M+H]⁺.

Step 6. LiOH (50 mg, 1.20 mmol, 1.5 eq) was added to a solution of Boc-Cit-Lys(Fmoc-D-Arg(Pbf))-Tyr-OMe (1.00 g, 0.80 mmol, 1.0 eq) in THF/water (1:1, 10 mL) at rt. After stirring at rt for 10 h, the organic solvent was concentrated then the aqueous solution was adjusted to pH 2-3 with citric acid. The precipitate was collected by filtration, washed with water, and purified on a Biotage Isolera One (C18 column, eluting with 5% to 95% ACN/water containing 0.1% FA) to provide Boc-Cit-Lys(D-Arg(Pbf))-Tyr-OH (0.40 g, 0.37 mmol, 90% UV purity, 46% yield) as a white solid. LC-MS (method 5): Rt = 0.98 min, m/z = 975.4 [M+H]⁺.

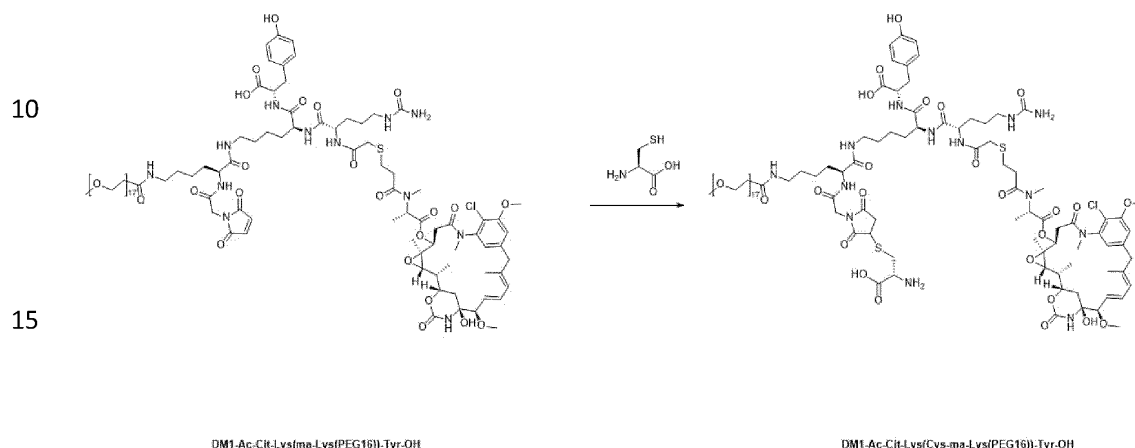
Step 7. Triethylamine (15 mg, 0.15 mmol, 1.5 eq) was added to a mixture of Boc-Cit-Lys(D-Arg(Pbf))-Tyr-OH (100 mg, 0.10 mmol, 1.0 eq), AMAS (28 mg, 0.11 mmol, 1.1 eq) in DMF (1 mL) at rt. After stirring at rt for 30 min, TFA was added until pH 5-6 was reached. Purification on a Biotage Isolera One (C18 column, eluting with 5% to 95% ACN/water containing 0.1% TFA) afforded Boc-Cit-Lys(D-Arg(Pbf)-ma)-Tyr-OH (38 mg, 29 μmol, 88% UV purity, 29% yield) as a white solid. LC-MS (method 6): Rt = 1.70 min, m/z = 1112.6 [M+H]⁺.

Step 8. Boc-Cit-Lys(D-Arg(Pbf)-ma)-Tyr-OH (10 mg, 9.0 μmol, 1.0 eq) was solubilized in a mixture of TFA/DCM (1:1, 1 mL) at rt. After stirring at rt for 30 min, the mixture was concentrated, washed with Et₂O and triturated in Et₂O to provide H-Cit-Lys(D-Arg-ma)-Tyr-OH (6.0 mg, 6.2 μmol, 90% UV purity, 69% yield) which was used directly in next step without further purification. LC-MS (method 6): Rt = 0.97 min, m/z = 758.4 [M+H]⁺.

Step 9. HOSu (1.6 mg, 13.8 μmol, 1.1 eq) was added to a mixture of DM1-Ac (10 mg, 12.6 μmol, 1.0 eq) and EDCI (2.6 mg, 13.8 μmol, 1.1 eq) in DMF (1 mL) at rt. After stirring at rt for 1 h, the reaction mixture was poured into water (40 mL) and extracted with EA (3 x 20 mL). The combined organic phases were dried over sodium sulfate and concentrated under vacuum. The residue was dissolved in DMF (1 mL). H-Cit-Lys(D-Arg-ma)-Tyr-OH (11.4 mg, 12.6 μmol, 1.0 eq) and DIEA (2.44 mg, 18.9 μmol,

1.5 eq) were added to the later solution at rt. After stirring at rt for 1 h, TFA was added until pH 5-6 was reached. Purification on a Biotage Isolera One (C18 column, eluting with 5% to 75% ACN/water containing 0.1% TFA) afforded DM1-Ac-Cit-Lys(D-Arg-ma)-Tyr-OH (3.0 mg, 2.27 μ mol, 88% UV purity, 18% yield) as a white solid. LC-MS (method 5): Rt = 1.01 min, m/z = 1537.5 [M+H]⁺.

Example 10.4.8 Preparation of DM1-Ac-Cit-Lys(Cys-ma-Lys(PEG16))-Tyr-OH



20 Cysteine (9.15 mg, 75.5 μ mol, 2.0 eq.) was added to a solution of DM1-Ac-Cit-Lys(ma-Lys(PEG16))-Tyr-OH (86.9 mg, 37.8 μ mol, 1.0 eq.) in DMF (7.6 mL) at rt. After stirring at rt for 5 h, the reaction mixture was filtered using a 33 mm 0.22 μ m hydrophobic filter. Purification by preparative HPLC (10 to 50% of ACN+0.1%TFA in water+0.1%TFA) afforded DM1-Ac-Cit-Lys(Cys-ma-Lys(PEG16))-Tyr-OH (62.4 mg, 25.8 μ mol, 100% UV purity, 68% yield) as a white powder after freeze-drying. UPLC-MS (method 1): Rt = 1.34 min, m/z = 1210 [M-2H]²⁻.

10.5 Preparation and characterisation of conjugate compounds, also referred to as Antibody-Drug conjugates (ADCs):

10.5.1 Preparation of ADC

General procedure for naratuximab conjugation (Targeted DAR8):

35 A solution of TCEP.HCl (1.10 mg, 3.80 μ mol, 8.0 eq.) in DPBS (0.11 mL) was added to a solution of naratuximab (70 mg, 0.48 μ mol, 1.0 eq.) in buffer (7.00 mL, 50 mM potassium phosphate, 50 mM potassium chloride, 2 mM EDTA, pH 6.5) at rt. The reaction mixture was purged with nitrogen then stirred at 40 °C. After stirring at 40 °C for 70 min, a solution of linker-payload, e.g., as specified in Table 2 below, (9.60

μmol, 20.0 eq.) in DMSO (0.7 mL) was added. The reaction mixture was stirred at rt for 70 min then diluted to V = 10 mL with 10x pH8 DPBS. Purification using a PF100 column and pH8 DPBS as eluent afforded a fraction containing the desired ADC (14 mL). This fraction was stirred at rt for 16 h then centrifugated (10 min, 4000 rpm) and finally the supernatant was transferred to an Amicon concentrating cell (15 mL, 50 kDa). The mixture was concentrated by centrifugation (4500 rpm, 3800 G) to V = 1 mL, DPBS buffer was added (14 mL) and the mixture was concentrated again (4500 rpm, 3800 G) to V = 1 mL. DPBS buffer was added (14 mL) and the mixture were concentrated again (4500 rpm, 3800 G) to V = 1 mL. The final volume was adjusted to V = 7.0 mL with DPBS buffer. The solution was filtered using a 25 mm PES 0.22 μm Millex filter then aliquoted and stored at -80 °C.

General procedure for naratuximab conjugation (Targeted DAR4):

A solution of TCEP.HCl (0.24 mg, 0.8 μmol, 2.5 eq.) in DPBS (0.245 mL) was added to a solution of naratuximab (50 mg, 0.34 μmol, 1.0 eq.) in buffer (5.00 mL, 50 mM potassium phosphate, 50 mM potassium chloride, 2 mM EDTA, pH 6.5) at rt. The reaction mixture was purged with nitrogen then stirred at 40 °C. After stirring at 40 °C for 70 min, a solution of linker-payload, e.g., as specified in Table 2 below, (4.5 mg, 2.7 μmol, 8 eq.) in DMF (0.5 mL) was added. The reaction mixture was stirred at rt for 60 min then diluted to V = 10 mL with 10x pH8 DPBS. Purification using a PF100 column and pH8 DPBS as eluent afforded a fraction containing the desired ADC (14 mL). This fraction was stirred at rt for 16 h then centrifugated (10 min, 4000 rpm) and finally the supernatant was transferred to an Amicon concentrating cell (15 mL, 50 kDa). The mixture was concentrated by centrifugation (4500 rpm, 3800 G) to V = 1 mL, DPBS buffer was added (14 mL) and the mixture was concentrated again (4500 rpm, 3800 G) to V = 1 mL. DPBS buffer was added (14 mL) and the mixture were concentrated again (4500 rpm, 3800 G) to V = 1 mL. The final volume was adjusted to V = 5.0 mL with DPBS buffer. The solution was filtered using a 25 mm PES 0.22 μm Millex filter then aliquoted and stored at -80 °C.

10.5.2 Summary of characterisation of prepared ADC

ADC number	mAb	Linker name	Targeted DOC/DAR
ADC-4	Naratuximab	DM1-Ac-Cit-Lys(ma-Lys(PEG16))Tyr-OH	8 / 8
N-DM1	Naratuximab	DM1-Ac-Cit-Lys(ma-PEG5)-Tyr-OH	4 / 4

Table 2: Summary of prepared ADCs

- 5 All characterisations are given in Table 3 below.

ADC number	Yield (%)	Concentration (mg/mL)	DAR by RPLC	Aggregation (%) by SEC after a freeze/thaw cycle
ADC-4	89.1	9.11	7.66	1.61
N-DM1	86	8.55	4.32	1.12

Table 3: Characterisations of prepared ADC

10.5.3 Preparation of naratuximab emtansine (Debio 1562)

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Naratuximab was reacted with the heterobifunctional crosslinking reagent SMCC and the maytansinoid DM1 using the one step process described in WO2012/135517 A2. The DAR was 3.5 (measured by UV at 2 different wavelengths 280 nm and 252 nm).

- 15 **10.6 Activity assessment of conjugate compounds when administered as monotherapy:**

10.6.1 *In vitro* cytotoxicity on DLBCL cell lines panel

- 20 A3/KAW, DOHH-2, HBL-1, KARPAS-422, OCI-LY19, OCI-LY3, OCI-LY7, Pfeiffer, U-DHL-2, SU-DHL-4, SU-DHL-5, SU-DHL-6, SU-DHL-8, Toledo, U-2932, WSU-DLCL2 and WSU-NHL cells were plated on day 0 and on day 1 serial dilutions of ADC-4, Debio 1562 or naratuximab (nine 10-fold dilutions, from 1 μ M down to 0.01 pM in triplicate) were added to the cells. After 72 hours of incubation, plates were inspected under an
- 25 inverted microscope to ensure growth of the controls and sterile conditions. Then, 50 μ L CellTiter Glo (Promega, G7572) were added prior to luminescence reading as per the manufacturer's instructions. Relative IC₅₀ were calculated using GraphPad Prism. IC₅₀ values of ADC-4, Debio 1562 and naratuximab are shown in Table 4.

Cell line	ADC-4 IC50 (nM)	Debio 1562 IC50 (nM)	Naratuximab IC50 (nM)
OCI-LY3	0,011	0,352	>1000
DOHH-2	0,018	0,949	>1000
WSU-NHL	0,019	0,190	>1000
SU-DHL-5	0,022	0,901	>1000
SU-DHL-4	0,035	0,983	>1000
SU-DHL-6	0,041	1,743	>1000
HBL-1	0,055	0,217	>1000
WSU-DLCL2	0,063	9,593	>1000
U-2932	0,113	3,572	>1000
SU-DHL-2	0,307	7,777	>1000
OCI-LY7	0,514	79,326	>1000
KARPAS-422	0,523	20,456	>1000
SU-DHL-8	1,844	21,503	>1000
Pfeiffer	2,422	73,486	>1000
Toledo	19,335	90,001	>1000
A3 /KAW	46,462	32,986	>1000
OCI-LY19	57,824	45,519	>1000

Table 4

10.7 Activity of combinations comprising conjugate compound derived from naratuximab and DM1-Ac-Cit-Lys(ma-PEG5)-Tyr-OH (referred to as N-DM1)

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10.7.1 Objective and outline of the experiment

The objective was to evaluate the cytotoxic activity of N-DM1 on the OCI-LY7 cell line (DLBCL) in novel combinations with pictilisib (PI3K inhibitor) or navitoclax (BCL2 inhibitor).

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10.7.2 Methods

OCI-LY7 cells were plated on day 0 and on day 1 were incubated in 96-well plates at 37 °C with 5% CO₂ using a 6x6 dose combination matrix with serial dilutions of N-DM1 (6 * 10-fold dilutions, from 1 µM down to 10 pM in triplicate) in combination with either pictilisib or navitoclax (6 * 10-fold dilutions, from 10 µM down to 100 pM in triplicate). After 72 hours of incubation (37 °C with 5% CO₂), 50µL CellTiter Glo (Promega, G7572) were added per well prior to luminescence reading as per the manufacturer's instructions. CrownSyn software was used to analyze and display the synergistic effect of the obtained experimental data via the Loewe additivity model (Loewe S. (1953) The

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problem of synergism and antagonism of combined drugs. Arzneimittelforschung,3,286-290.) and the Bliss independence model (Bliss C.I. (1939) The toxicity of poisons applied jointly. Ann. Appl. Biol .,26,585-615).

5 10.7.3 Results

Both combinations of N-DM1 with Pictilisib or Navitoclax in the OCI-Ly7 cell line displayed synergy across multiple dose combinations that was consistent across both synergy analysis methods (Loewe and Bliss). The individual dose combination synergy
10 scores are displayed in Tables 5 to 8 below (values of 5 and above being indicative of significant synergy) and in Figures 1 and 2, with all concentrations being in nM unit.

These findings are indicative of robust synergy for both the combination of N-DM1 with Pictilisib or Navitoclax in the DLBCL cell line OCI-Ly7.

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N-DM1 combined with Pictilisib in the OCI-Ly7 cell line

Pictilisib/N-DM1	0.00000	0.01000	0.10000	1.00000	10.00000	100.00000	1000.00000
0	0	0	0	0	0	0	0
0.1	0	19.02	2.73	8.96	-0.2	1.45	1.44
1	0	0.43	1.43	7.7	0.81	2.67	1.42
10	0	-4.48	4.94	15.16	5.98	6.76	1.29
100	0	2.47	24.99	41.24	33.09	26.35	0.47
1e+03	0	-0.01	42.14	48.09	44.79	32.97	-7.27
1e+04	0	3.53	28.8	32.72	33.15	32.92	-27.31

Table 5: Synergy scores (Loewe)

Pictilisib/N-DM1	0.00000	0.01000	0.10000	1.00000	10.00000	100.00000	1000.00000
0	0	0	0	0	0	0	0
0.1	0	19.2	-1.05	-0.26	4.28	-0.45	0.75
1	0	6.37	2.86	2.7	9.15	2.63	0.82
10	0	-2.2	2.94	7.35	11.7	5.4	0.76
100	0	5.86	23.06	33.09	37.78	23.87	1.04
1e+03	0	1.37	38.55	36.08	34.01	16.59	0.64
1e+04	0	4.45	26.39	24.32	22.73	11.07	0.48

20 Table 6: Synergy scores (Bliss)

N-DM1 combined with Navitoclax in the OCI-Ly7 cell line

Navitoclax /N-DM1	0.00000	0.01000	0.10000	1.00000	10.00000	100.00000	1000.00000
0	0	0	0	0	0	0	0
0.1	0	0.71	7.54	9.2	-0.81	0.01	3.1
1	0	-1.7	6.99	10.6	-2.02	1.34	3.04
10	0	4.74	5.82	9.36	1.22	0.23	2.98
100	0	-0.96	11.06	10.1	-0.25	1.34	2.96
1e+03	0	-2.41	9.33	11.81	2.96	5.44	2.19
1e+04	0	1.51	0.62	1.09	1.32	-2.88	-13.95

Table 7: Synergy scores (Loewe)

Navitoclax /N-DM1	0.00000	0.01000	0.10000	1.00000	10.00000	100.00000	1000.00000
0	0	0	0	0	0	0	0
0.1	0	8.19	7.65	7.77	8.79	2.41	0.6
1	0	6.47	7.65	9.6	7.96	3.93	0.55
10	0	11.01	4.94	7.15	10.14	2.3	0.48
100	0	2.87	8.1	6.23	7.2	2.72	0.51
1e+03	0	1.35	5.11	6.66	9.04	6.57	0.6
1e+04	0	1.44	-0.08	-0.09	0.66	0.24	-0.03

Table 8: Synergy scores (Bliss)

5

10.8 Activity of combinations comprising Debio 1562 as the conjugate compound

10.8.1 Objective and outline of experiment

10

The objective was to determine the cytotoxic activities of novel combination therapies comprising the antibody drug conjugate (ADC) Debio 1562.

15

Debio 1562 consists of an antibody that recognizes CD37, covalently linked to the microtubule poison DM1. CD37 is a cell surface molecule present on many B cell malignancies that include diffuse large B cell lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia and follicular lymphoma. Tumor cell killing may occur by a variety of mechanisms that include complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) and delivery of DM1 to tumor cells.

20

Debio 1562 was combined with BCL2 inhibitors and PI3K pathway inhibitors and tested across a panel of 13 cell lines using an 8x6 combination dose response matrix over a treatment time of seventy-two hours.

10.8.2 Methods

Anti-proliferation Assay

5 Cells are thawed from a liquid nitrogen preserved state. Once cells have been expanded and divide at their expected doubling times, screening begins. Cells are seeded in growth media in black 384- or 1536-well tissue culture treated plates. Cells are equilibrated in assay plates via centrifugation and placed in incubators attached to the Dosing Modules at 37°C for twenty-four hours before treatment. At the time of
10 treatment, a set of assay plates (which do not receive treatment) are collected and ATP levels are measured by adding ATPLite (Perkin Elmer). These Tzero (To) plates are read using ultra-sensitive luminescence on Envision Plate Readers. Treated assay plates are incubated with compound for seventy-two hours. After seventy-two hours, plates are developed for endpoint analysis using ATPLite.

15 Assay plates are accepted if they pass the following quality control standards: relative luciferase values are consistent throughout the entire experiment, Z-factor scores are greater than 0.6, untreated/vehicle controls behave consistently on the plate. The calculation for Synergy Score is provided below.

20 Growth Inhibition (GI) is used as a measure of cell viability. The cell viability of vehicle is measured at the time of dosing (To) and after seventy-two hours (T72). A GI reading of 0% represents no growth inhibition - cells treated with compound and T 72 vehicle signals are matched. A GI 100% represents complete growth inhibition - cells treated
25 by compound and To vehicle signals are matched. Cell numbers have not increased during the treatment period in wells with GI 100% and may suggest a cytostatic effect for compounds reaching a plateau at this effect level. A GI 200% represents complete death of all cells in the culture well.

30 Compounds reaching an activity plateau of GI 200% are considered cytotoxic. GI is calculated by applying the following test and equation:

$$\text{If } T < V_0 : 100 * (1 - \frac{T-V_0}{V_0})$$

$$\text{If } T \geq V_0 : 100 * (1 - \frac{T-V_0}{V-V_0})$$

35 where T is the signal measure for a test article, V is the vehicle-treated control measure, and Vo is the vehicle control measure at time zero. This formula is derived

from the Growth Inhibition calculation used in the National Cancer Institute's NCI-60 high throughput screen. For the purposes of this experiment, all combination data analysis were performed in growth inhibition.

- 5 The experiment also provides Inhibition as a measure of cell viability. Inhibition levels of 0% represent no inhibition of cell growth by treatment. Inhibition of 100% represents no doubling of cell numbers during the treatment window. Both cytostatic and cytotoxic treatments can yield an Inhibition Percentage of 100%. Inhibition Percentage is calculated as the following:

10

$$I = 1 - T/U$$

Where T is the treated and U is the untreated.

15 Synergy Score Analysis

To measure combination effects in excess of Loewe additivity, a scalar measure is devised to characterize the strength of synergistic interaction termed the Synergy Score. The Synergy score is

20 calculated as:

$$\text{Synergy Score} = \log f_X \log f_Y \sum \max(0, I_{\text{data}}) (I_{\text{data}} - I_{\text{Loewe}})$$

The fractional inhibition for each component agent and combination point in the matrix is calculated relative to the median of all vehicle-treated control wells. The Synergy Score equation integrates the experimentally-observed activity volume at each point in the matrix in excess of a model surface numerically derived from the activity of the component agents using the Loewe model for additivity. Additional terms in the Synergy Score equation (above) are used to normalize for various dilution factors used for individual agents and to allow for comparison of synergy scores across an entire experiment. The inclusion of positive inhibition gating or an I_{data} multiplier removes noise near the zero effect level, and biases results for synergistic interactions at that occur at high activity levels. Combinations with higher maximum Growth Inhibition (GI) effects or those which are synergistic at low concentrations will have higher Synergy Scores.

35

Loewe Volume Score Analysis

Loewe Volume is used to assess the overall magnitude of the combination interaction in excess of the Loewe additivity model. Loewe Volume is particularly useful when distinguishing synergistic increases in a phenotypic activity (positive Loewe Volume) versus synergistic antagonisms (negative Loewe Volume). When antagonisms are observed, as in the current dataset, the Loewe Volume should be assessed to examine if there is any correlation between antagonism and a particular drug target-activity or cellular genotype. This model defines additivity as a non-synergistic combination interaction where the combination dose matrix surface should be indistinguishable from either drug crossed with itself.

The calculation for additivity is:

$$f_{\text{Loewe}} \text{ that satisfies } (X/X_i) + (Y/Y_i) = 1$$

where X_i and Y_i are the single agent effective concentrations for the observed combination effect I . For example, if 50% inhibition is achieved separately by 1 μ M of drug A or 1 μ M of drug B, a combination of 0.5 μ M of A and 0.5 μ M of B should also inhibit by 50%.

Activity observed in excess of Loewe additivity identifies potential synergistic interaction. For the present analysis, empirically derived combination matrices were compared to their respective Loewe additivity models constructed from experimentally collected single agent dose response curves. Summation of this excess additivity across the dose response matrix is referred to as Loewe Volume. Positive Loewe Volume is indicative of synergy. Negative Loewe Volume is indicative of antagonism.

Combination Screen Design

The experiment examined Debio 1562 activity in combination with BCL2 inhibitors or PI3K pathway inhibitors. The combination screen was performed in 13 cell lines representative of diffuse large B cell lymphoma (DLBCL, both the activated B cell subtype (ABC) and the germinal B cell (GCB) subtype).

Combination data was collected in an 8x6 optimized matrix for 13 cell lines screened in a 384 or 1536-well format. The combination partners selected from BCL2 inhibitors and PI3K pathway inhibitors (sometimes referred to as enhancer compounds) were combined with the conjugate compound (sometimes referred to as enhancee) across

the 13 cell line panel. The starting concentration and fold dilution for the enhancee and enhancer compounds were selected as shown in the following Table 9.

Cell lines	Top Screening conc. μM	Titration Factor (fold dilution)	No. of Dose Levels Tested
OCI-Ly7	0.12	3	7
OCI-Ly10, SU-DHL-10-epst, U-2932	3.6×10^{-2}	3	7
DOHH-2, HBL-1, OCI-Ly1, SU-DHL-2-epst, SU-DHL-6-epst, TMD8	4.0×10^{-3}	3	7
RL, SU-DHL-4-epst, WSU-NHL	2.0×10^{-3}	2	7

Table 9

5

10.8.3 Results on combinations comprising PI3K Pathway Inhibitors

Cytotoxic activity was observed across three nodes in the PI3K pathway, namely: PI3K, AKT and mTOR. Inhibitors of these signaling nodes in combination with Debio
10 1562 led to pronounced synergy in the majority of the cell lines.

The results of the synergy score calculations are shown in the following Table 10.

	HBL-1	OCI-Ly10	SU-DHL-2-epst	TMD8	U-2932	DOHH-2	OCI-Ly1	OCI-Ly7	RL
IPI-145	7.5	5.9	1.1	3.4	3.5	32.4	4.0	19.2	1.5
CAL-101	5.5	5.1	0.3	7.7	4.2	24.8	11.1	22.9	4.3
IC-87114	3.4	3	0.4	11.4	3.9	11.2	3.6	10.3	0.7
PIK-93	10.9	4.3	5.7	1.0	28.4	15.6	6.1	17.0	3.3
GDC-0941	11.9	3.8	6.1	5.2	12.1	23.8	7.5	31.8	3.3
BEZ235	4.4	4	6.9	0.2	2.7	12.0	5.6	16.1	0.5
GSK1059615	14.3	3.8	3.8	3.7	19.0	12.4	6.1	11.7	5.6
Everolimus	1.5	5.8	7.1	4.4	4.6	2.7	2.4	5.2	1.2
Temsirolimus	1.2	5.7	7.6	7.2	5	6.4	1.6	8.8	0.7
KU0063794	5.8	4.1	10.4	0.4	8.7	23	4	25.4	5.8
AZD8055	6.9	5.9	11.4	0.6	8.8	25.6	6	30.8	4.6
AT7867	1	1.4	0	1.7	25.3	11	2.6	5.7	0.3
MK-2206	2.6	2.4	3.7	8.5	20	32.2	8.9	28.1	10.7
Perifosine	3.4	0.5	1.2	1.2	6.8	0.5	1.6	5.2	0.4
Triciribine	9.7	10.4	7.5	7.2	30.3	8.5	3.8	3.9	0.9
BX-912	0.7	0.1	0.2	1.2	2.3	0.4	2.1	3.4	0.3

Table 10

15

	SU-DHL-10-epst	SU-DHL-4-epst	SU-DHL-6-epst	WSU-NHL
IPI-145	6.6	20.2	1.0	20.1
CAL-101	8.3	21.1	0.6	30.8
IC-87114	3.6	9.5	0.6	13.0
PIK-93	6.9	9.3	0.9	14.5
GDC-0941	5.6	1.5	0.4	16.1
BEZ235	5.0	9.3	0.5	2.6
GSK1059615	3.1	10.1	2.3	8.8
Everolimus	6.1	3	1.1	0.4
Temsirolimus	5.8	9.4	1.4	0.5
KU0063794	7.9	22.7	1.6	8.7
AZD8055	17	16.6	5.2	13.2
AT7867	8.7	6.1	0.1	4.3
MK-2206	8.4	6.5	8.1	23.8
Perifosine	6.7	1.7	0.2	0.9
Triciribine	1.3	3.3	0.8	3.5
BX-912	0.4	1.3	0.2	

Table 10 (continued)

For the class of compounds reported in Table 10, synergy scores of 6.0 or higher are considered to be statistically significant. A considerable number of statistically significant synergy scores was found.

Furthermore, the combination of ADC-4 with idelalisib (CAL-101) led to pronounced synergy in the majority of the cell lines.

	Ramos	U-2932	Toledo	RL	SU-DHL-4	Pfeiffer	SU-DHL-8
CAL-101	9.8	9.4	8.0	6.8	6.2	4.9	4.4

10 Table 10a

For the compound reported in Table 10a, synergy scores of 5.0 or higher are considered to be statistically significant.

15 10.8.4 Results on combinations comprising BCL-2 Family Inhibitors

Combinations of Debio 1562 with BCL2 inhibitors also showed synergistic cytotoxic effects. The results are summarized in the following Table 11.

	HBL-1	OCI-Ly10	SU-DHL-2-epst	TMD8	U-2932	DOHH-2	OCI-Ly1	OCI-Ly7	RL
ABT-199	18.4	2.6	1.2	0.7	21.3	22.6	4.4	7.3	10.1
ABT-263	20.7	13.2	7.8	2.9	19.7	20.9	4.0	8.4	12.7
ABT-737	20.1	31.2	11.3	9	17	20.5	6.5	7.3	17.6

Obatoclax Mesylate	2.0	0.4	0.7	1.3	4.3	2.2	2.9	3.8	0.1
TW-37	8.6	2.3	1.7	1.3	10.4	4.2	4.4	11.5	4.6

Table 11

	SU-DHL-10-epst	SU-DHL-4-epst	SU-DHL-6-epst	WSU-NHL
ABT-199	0.9	18.2	0.1	
ABT-263	3.8	12.1	20.9	13.3
ABT-737	4.0	21.4	27.5	17.5
Obatoclax Mesylate	6.4	0.0	0.1	4.2
TW-37	8	1.9	2.4	2.3

Table 11 (continued)

- 5 For the class of compounds reported in Table 11, synergy scores of 7.3 or higher are considered to be statistically significant. Statistical significance was reached by a considerable number of data points.

- 10 Furthermore, the combination of ADC-4 with venetoclax (ABT-199) led to pronounced synergy in the majority of the cell lines.

	Ramos	U-2932	Toledo	RL	SU-DHL-4	Pfeiffer	SU-DHL-8
ABT-199	2.3	6.6	11.6	7.6	4.7	3.2	5.7

Table 11a

- 15 For the compound reported in Table 11a, synergy scores of 5.0 or higher are considered to be statistically significant.

10.9 Cytotoxic activity of combinations and reversal of resistance against conjugate compound naratuximab emtansine (Debio 1562) monotherapy

20 10.9.1 Overview

- 25 After long exposure to the naratuximab emtansine ADC (Debio 1562), the SU-DHL-4 DLBCL cell line developed resistance to naratuximab emtansine through, for example, an activating mutation in the PIK3CD gene, associated with increased sensitivity to PI3K δ inhibition and a switch from functional dependence on the anti-apoptotic protein MCL1 to dependence on BCL2. The addition of idelalisib or venetoclax to naratuximab emtansine overcame resistance to the ADC in the resistant derivative while also improving the cytotoxic activity of the ADC in the parental cells.

10.9.2 Materials and methods

Cell line and Compounds

The DLBCL cell line SU-DHL-4 was used in this study. Naratuximab emtansine was provided by Immunogen and Debiopharm. Targeted agents idelalisib (PI3K δ inhibitor) and venetoclax (BCL2 inhibitor) were purchased from Selleckchem.

Proliferation upon single and combination treatments

SU-DHL-4 cells were seeded in 96-well plates, exposed to compounds added to create 4-fold dilution series ranging from 200 nM to 0.19 pM and assayed by MTT following 72 hours (h) of treatment, as previously described (e.g. in Hicks SW, Tarantelli C, Wilhem A, et al. The novel CD19-targeting antibody-drug conjugate huB4-DGN462 shows improved anti-tumor activity compared to SAR3419 in CD19-positive lymphoma and leukemia models. *Haematologica*. 2019;104(8):1633-1639). In one particular experiment, resistant and parental SU-DHL-4 were exposed for 72 hours to increasing concentrations of naratuximab emtansine after 72 hours pretreatment with DMSO or with 100 nM or 500 nM of idelalisib. Sensitivity to single drug treatments was evaluated by the IC₅₀ (4-parameters calculation upon log-scaled doses, R package calculation (Smirnov P, Safikhani Z, El-Hachem N, et al. *PharmacoGx: an R package for analysis of large pharmacogenomic datasets*. *Bioinformatics*. 2016;32(8):1244-1246)). The beneficial effect of the combinations versus the single agents was considered as synergism according to the Chou-Talalay combination index (Chou TC. *Drug combination studies and their synergy quantification using the Chou-Talalay method*. *Cancer Res*. 2010;70(2):440-446).

Development of resistant cell lines

SU-DHL-4 cell line was exposed to a concentration equal to approximately 25% of the naratuximab emtansine IC₅₀ dose, and incremental increase in dosing was performed at every passage (every 2-3 days). Parental (sensitive) cell line was cultured in parallel to resistant line with no drug exposure. SU-DHL-4 parental and resistant cells were cultured for 72hr in presence of naratuximab emtansine and proliferation was assessed using MTT proliferation assay. SUD-HL-4 parental and resistant lines were treated with idelalisib or venetoclax for 48hr and proliferation was assessed using MTT assay.

Whole exome sequencing (WES) and RNA-Seq

DNA and RNA sequencing were performed as described in 2 references from Arribas AJ, Napoli S, Cascione L, et al., namely (1) Resistance to PI3K δ inhibitors in marginal zone lymphoma can be reverted by targeting the IL-6/PDGFRA axis. *Haematologica*. 2022;107(11):2685-2697), or (2) ERBB4-mediated signaling is a

mediator of resistance to BTK and PI3K inhibitors in B cell lymphoid neoplasms. bioRxiv. 2023:2023.2001.2001.522017.

Functional annotation was performed using the Gene Set Enrichment Analysis (GSEA) with the Molecular Signatures Database (MSigDB) (see e.g. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102(43):15545-15550), and SignatureDB database (see e.g. Shaffer AL, Wright G, Yang L, et al. A library of gene expression signatures to illuminate normal and pathological lymphoid biology. Immunol Rev. 2006;210:67-85).

Data analyses

Moderated t-test (LIMMA R-package, see e.g. Smyth GK. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol. 2004;3:Article3) was performed to determine statistically significant differences ($p < 0.05$) in each experiment. Pearson correlation was evaluated using the R environment. Mann-Whitney test was performed to evaluate the association of IC₅₀ values and genetic lesions.

10.9.3 Results

Development of DLBCL cell lines with resistance to naratuximab emtansine

To gain insights of potential mechanisms of resistance to naratuximab emtansine, the ADC sensitive DLBCL SU-DHL-4 cell line was exposed to increasing concentrations of the drug starting from the IC₅₀ for several months until they acquired resistance to the CD37 targeting ADC. Parental cell line was also kept in culture with no drug exposure. After approximately seven months cells kept under drug developed resistance to naratuximab emtansine. The resistance was demonstrated to be stable, by treating cells after two weeks with no drug exposure. IC₅₀ values was 6- fold higher in resistant SU-DHL-4 than in their parental cells (Figure 3).

Activation of PI3K δ due to gene mutations as a mechanism of resistance to naratuximab emtansine

Transcriptome profiling in the resistant SU-DHL-4 compared to their parental cells showed an enrichment of gene sets involved in PI3K signaling, lipid metabolism and cell death. Resistant cells exhibited de-regulation of the BCL2-family genes. WES identified a series of mutations in the resistant cells, but only two variants were expressed at mRNA levels. One of them was represented by a heterozygous missense mutation in the *PIK3CD* gene coding for PI3K δ .

In view of the observed BCL2 family gene deregulation and PIK3CD gene mutation, the SU-DHL-4 resistant cells and their parental counterpart were exposed to the BCL2 inhibitor venetoclax and the PI3K δ inhibitor idelalisib. The resistant cells were much
5 more sensitive to both agents than their parental cells (Figure 4). Each of the two agents, when given in combination with naratuximab emtansine, was able to overcome resistance to the ADC. Moreover, adding venetoclax or idelalisib to the ADC was beneficial also in the parental cells (Figures 5 and 6, respectively). Also, pre-treatment
10 with idelalisib restored sensitivity to naratuximab emtansine in the SU-DHL-4 resistant cells (Figure 7).

CLAIMS

1. A combination comprising a conjugate compound of the following formula (I) or
 5 pharmaceutically acceptable salt, solvate or polymorph thereof, and a co-agent
 selected from a BCL2 inhibitor or pharmaceutically acceptable salt, solvate or
 polymorph thereof and a PI3K pathway inhibitor or pharmaceutically acceptable salt,
 solvate or polymorph thereof, wherein the formula (I) is



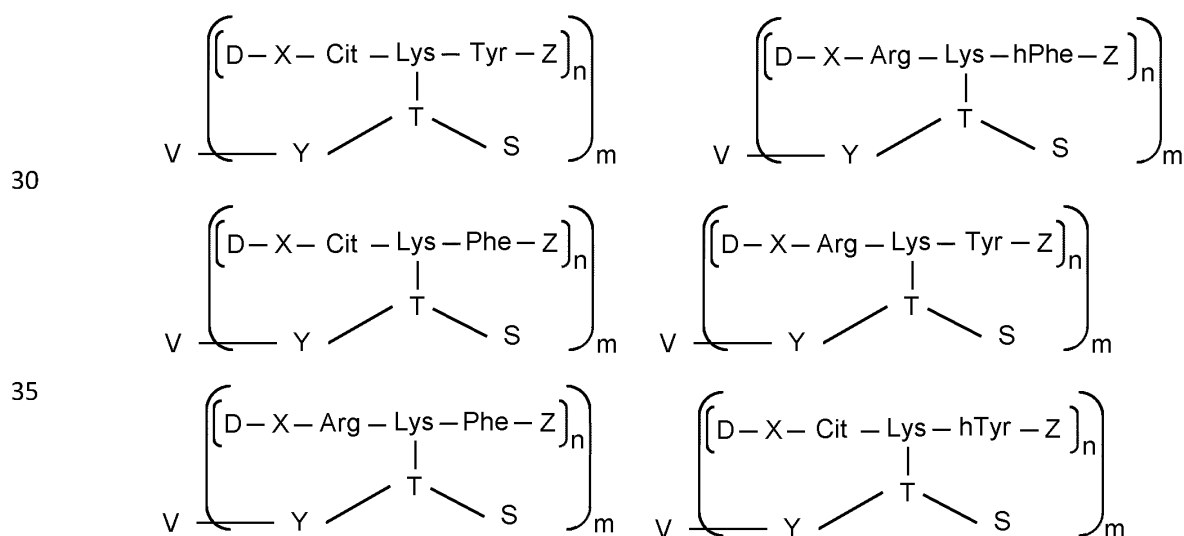
wherein V represents an anti-CD37 antibody, a fragment or derivative thereof or Fc-
 fusion protein containing an anti-CD37 antibody fragment and preferably naratuximab,
 a fragment or derivative thereof or Fc-fusion protein containing a naratuximab
 15 fragment,

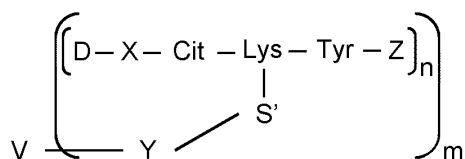
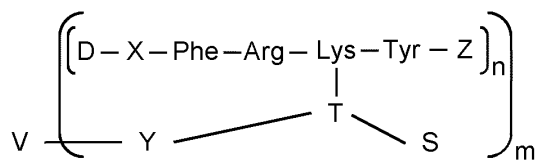
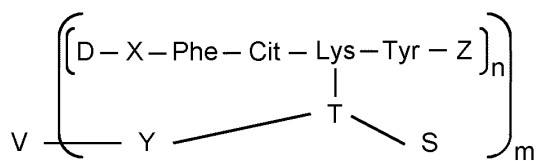
LL represents a linker covalently bonded to V and D,

D represents a moiety derived from a drug selected from maytansine, mertansine
 20 (DM1) and ravtansine (DM4), preferably DM1 or DM4 and more preferably DM1; and

m is an integer of 1 to 12, preferably 2 to 10 and more preferably 4 to 8.

2. The combination of claim 1, wherein the conjugate compound of formula (I) is a
 25 compound represented by one of the following formulae:

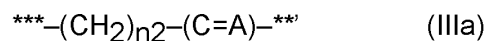




wherein

D has the same meaning as specified in claim 1, and preferably D is a moiety derived from DM1 or DM4;

X is a group of formula (IIIa),

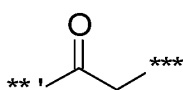


wherein n_2 is 1 or 2,

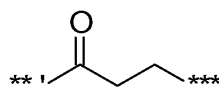
A is selected from O and S, preferably O;

*** represents covalent attachment to D; and

**' represents covalent attachment to the adjacent amino acid Arg, Cit or Phe; preferably a group represented by formula (IVc) or (IVd)



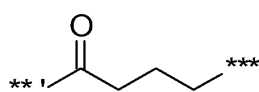
(IVc)



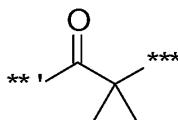
(IVd)

or

X is a group represented by formula (IVe) or (IVf)



(IVe)



(IVf)

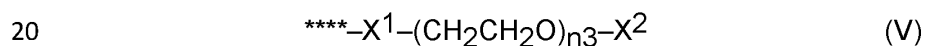
*** represents covalent attachment to D;

**' represents covalent attachment to the adjacent amino acid Arg, Cit or Phe;

Y is a group derived from a compound selected from maleimides, triazoles, hydrazones, carbonyl-containing compounds and derivatives thereof, preferably from maleimides and derivatives thereof such as opened hydrolyzed maleimide derivatives, and more preferably derived from an opened hydrolyzed maleimide;

T is derived from an amino acid selected from N-ε-propargyloxycarbonyl-L-Lysine (Lys(Poc)), Asp, Glu, Orn, Lys, Dab and Dap, preferably derived from Lys(Poc), Glu, Orn or Lys, more preferably derived from Lys;

S is a moiety of formula (V)



wherein

$n3$ is an integer of 6 to 200, preferably 10 to 150, more preferably 12 to 80 and in particular one or 12 to 30 or 14 to 25 or 15 to 19;

**** indicates covalent attachment to T, preferably the side chain of T;

X^1 is selected from a single covalent bond, $-(C=O)-$, and $-N(R)-$ in which R represents a hydrogen atom, an alkyl group or a cycloalkyl group;

X^2 represents an alkyl group having 1 to 6 carbon atoms, a carbonyl-containing group such as an acetyl group or a group of

5 formula $-(CH_2)_{n4}-CO_2H$, a thiocarbonyl-containing group, a group of formula $-(CH_2)_{n4}OR$, a group of formula $-(CH_2)_{n4}-SO_3H$, or an amino-containing group such as a group of formula $-(CH_2)_{n4}-(C=A)-N(R)_2$ or $-(CH_2)_{n4}-N(R)_2$, in which A is O or S, each R is independently selected from a hydrogen atom, an alkyl group and a cycloalkyl group, and n_4 is an integer of 1 to 6

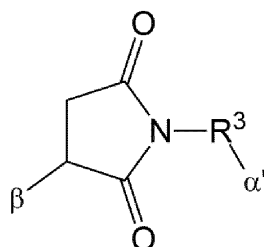
10 S' is a moiety represented by the following formula
 $\theta_1-(C=O)_{sn1}-(CH_2-CH_2-O)_{sn2}-(CH_2)_{sn3}-(NH)_{sn4}-\theta_2$

15 wherein sn_1 and sn_4 are independently selected from 0 and 1, sn_2 is selected from 2 to 8, preferably 3 to 7 and more preferably 4 to 6, and sn_3 is selected from 1 and 2, wherein most preferably sn_1 and sn_4 are each 1, sn_2 is 5, and sn_3 is 2, and wherein θ_1 is the position of a covalent bond to the side chain of Lys and θ_2 is the position of a covalent bond to Y;

20 Z is $-OH$; and
 n is 1.

3. The combination of claim 2, wherein group S in the conjugate compound of formula (I) is represented by a moiety of formula (V), wherein X^1 is $-(C=O)-$, X^2 is a methyl group and n_3 is selected from one or the ranges of 12 to 30, 14 to 25, 14 to 20, 15 to 19, and 16 to 18.

4. The combination of any one of claims 2 and 3, wherein group Y in the conjugate compound of formula (I) is represented by a divalent group represented by any of the following formula (XIIIa):



Formula (XIIIa)

wherein,

R^3 represents $-(CH_2)_{n7}-(C=A)_{n9}-\alpha'$ or $-(CH_2)_{n7}-(C=A)_{n9}-NH-(CH_2CH_2O)_{n8}-(C=A)_{n9}-\alpha'$, preferably $-(CH_2)_{n7}-(C=A)_{n9}-\alpha'$, wherein,

$n7$ is 1 or 2, preferably 1,

$n8$ is 1 to 6, preferably 1,

$n9$ is 0 or 1, preferably 1, and

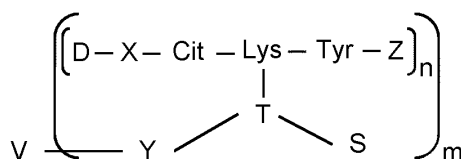
A is O or S, preferably O;

wherein the methylene carbon atom of R^3 is covalently attached to the nitrogen atom of formulae (XIIIa) and the carbonyl or thiocarbonyl-carbon is covalently attached to T;

β indicates covalent attachment to V; and

α' indicates covalent attachment to T, preferably by amide bond formation with the amino group that is attached to $C\alpha$ of the amino acid.

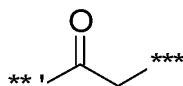
5. The combination of any one of claims 2, 3 and 4, wherein the conjugate compound of formula (I) is a compound of the following structure:



wherein D, X, Z, T, Y, S, V, n and m are as defined in any of the preceding claims, wherein preferably

D is a moiety derived from DM1;

X is a group represented by formula (IVc)



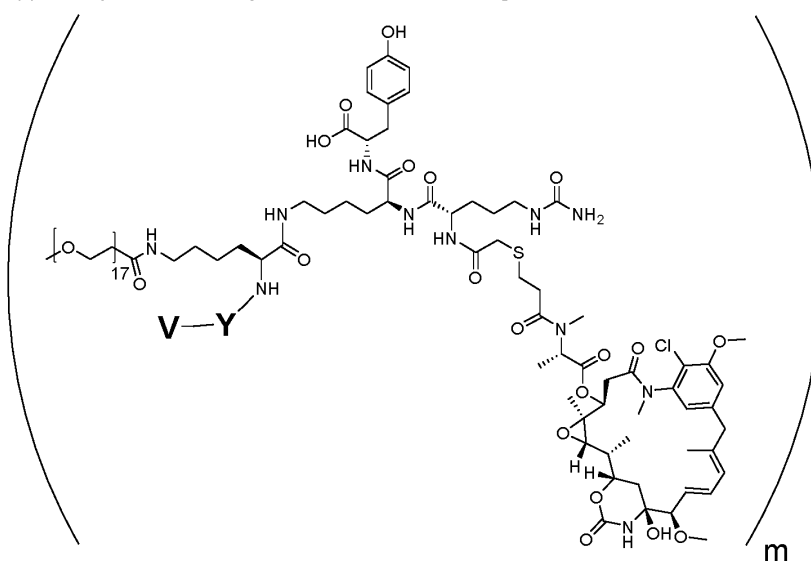
(IVc)

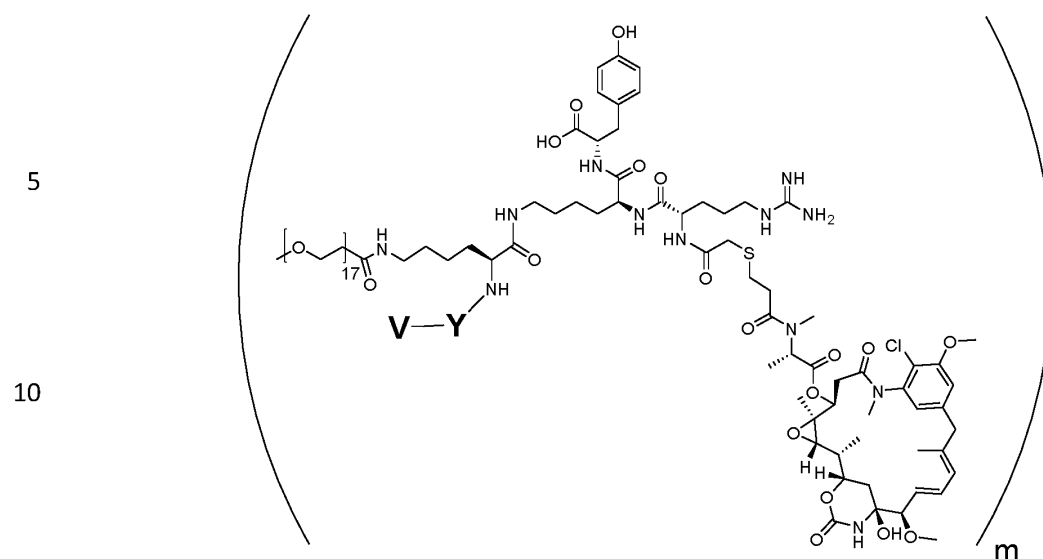
*** represents covalent attachment to D;

** represents covalent attachment to the adjacent amino acid Cit;

- Y is a group of formula (XIIIa) as defined in claim 4, wherein R^3 represents $-(CH_2)_{n7}-(C=A)_{n9}-\alpha'$, wherein,
 $n7$ is 1,
 $n9$ is 1, and
 A is O;
 wherein the methylene carbon atom of R^3 is covalently attached to the nitrogen atom of formula (XIIIa) and the carbonyl-carbon is covalently attached to T, forming an amide bond with the Lys;
 β indicates covalent attachment to V; and
 α' indicates covalent attachment to T, preferably by amide bond formation with the amino group that is attached to $C\alpha$ of the amino acid;
 T is derived from an amino acid selected from Lys(Poc), Glu, Orn or Lys, preferably derived from Lys;
 S is as specified in claim 3;
 Z is $-OH$;
 V is derived from naratuximab; and
 n is 1.

6. The combination of any one of claims 1 to 5, wherein the conjugate compound of formula (I) is represented by one of the following formulae:





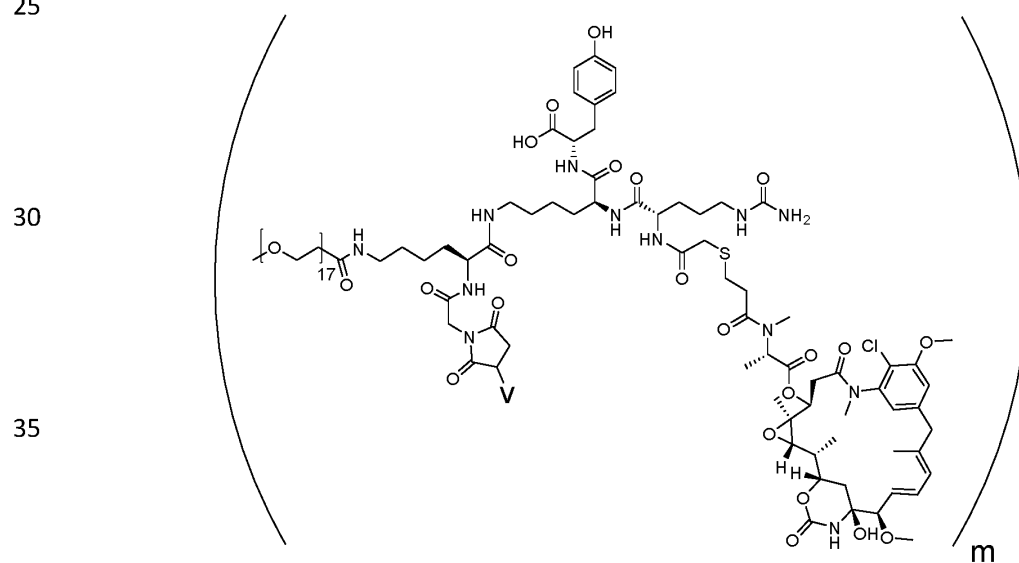
15 wherein Y, V and m are as defined in any of the preceding claims, preferably wherein Y is as defined in claim 4, V is naratuximab and m is 2-10

with the proviso that V is to be understood as being outside the parentheses, such that m does not apply to V and with the proviso that the number of oxyethylene groups may also be selected from the ranges 12 to 30, preferably 14 to 25, more preferably 15 to 19.

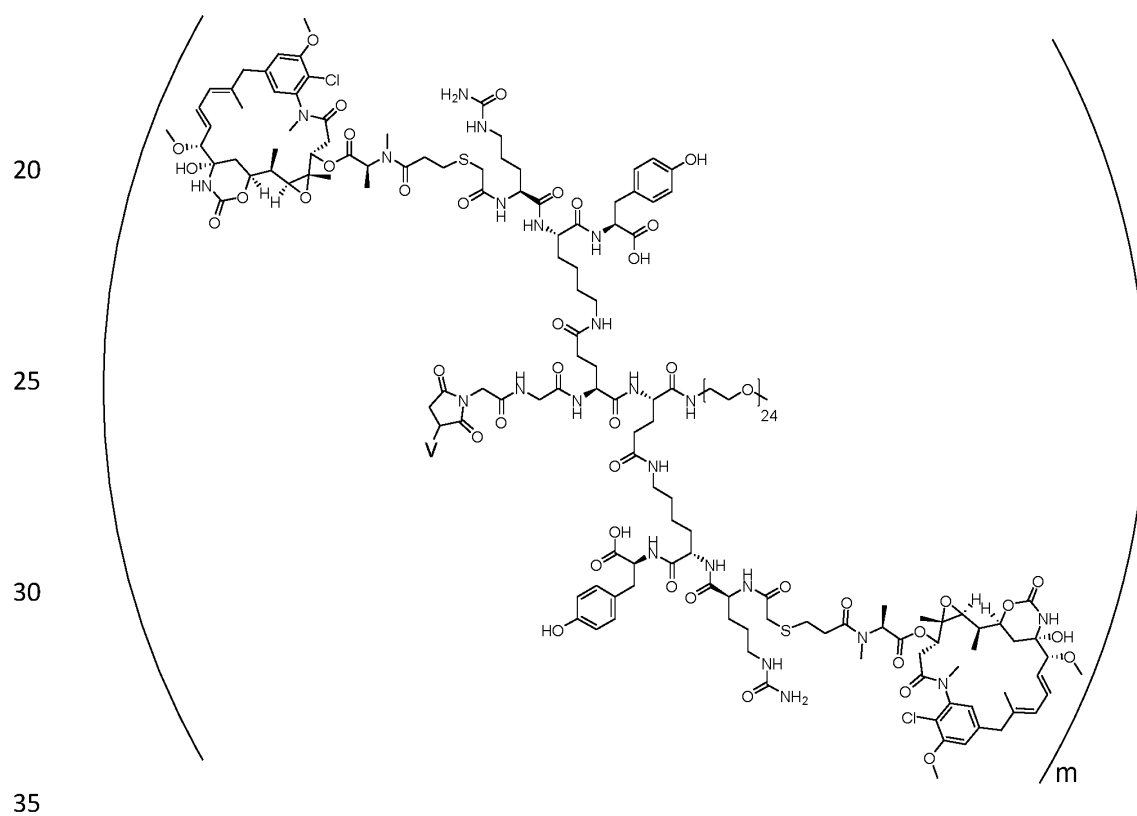
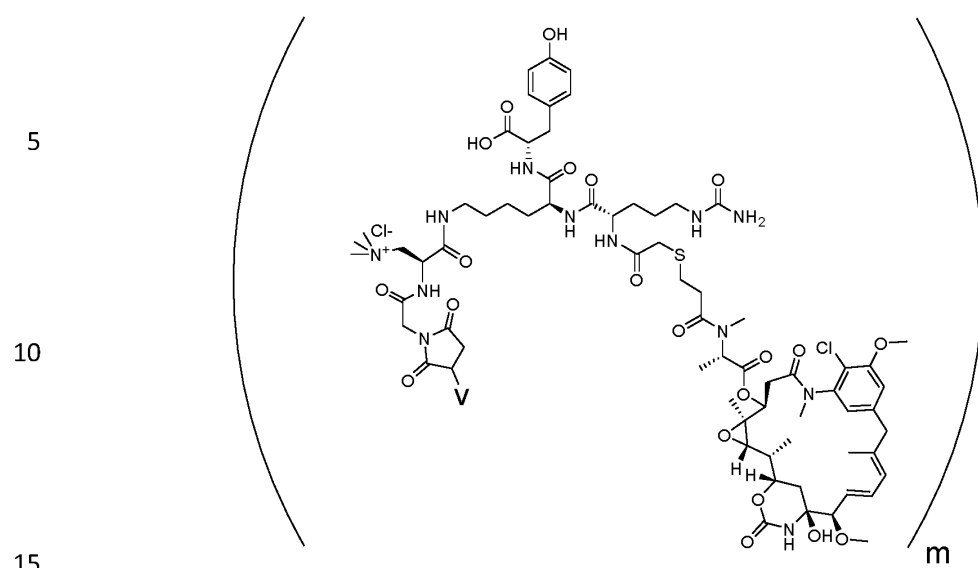
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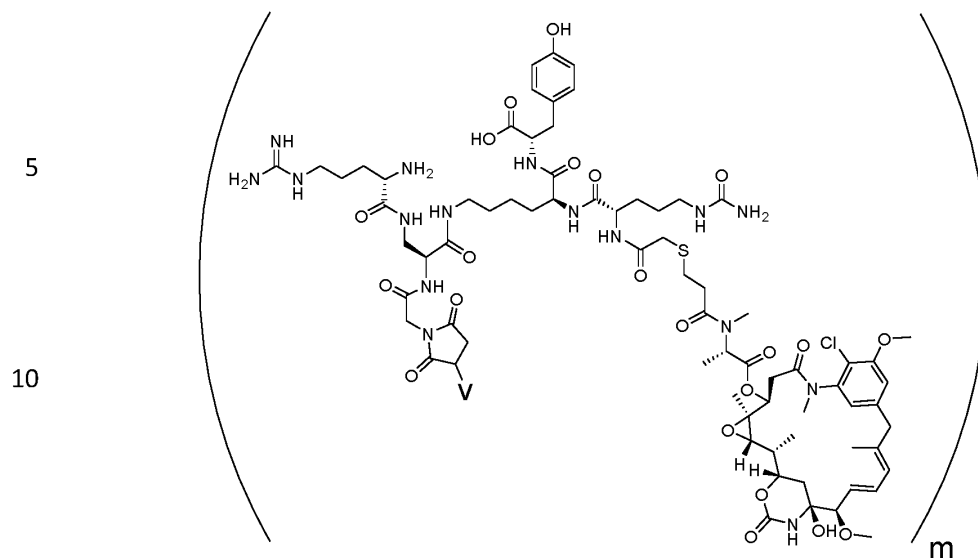
7. The combination of any one of claims 1 to 5, wherein the conjugate compound of formula (I) is represented by one of the following formulae:

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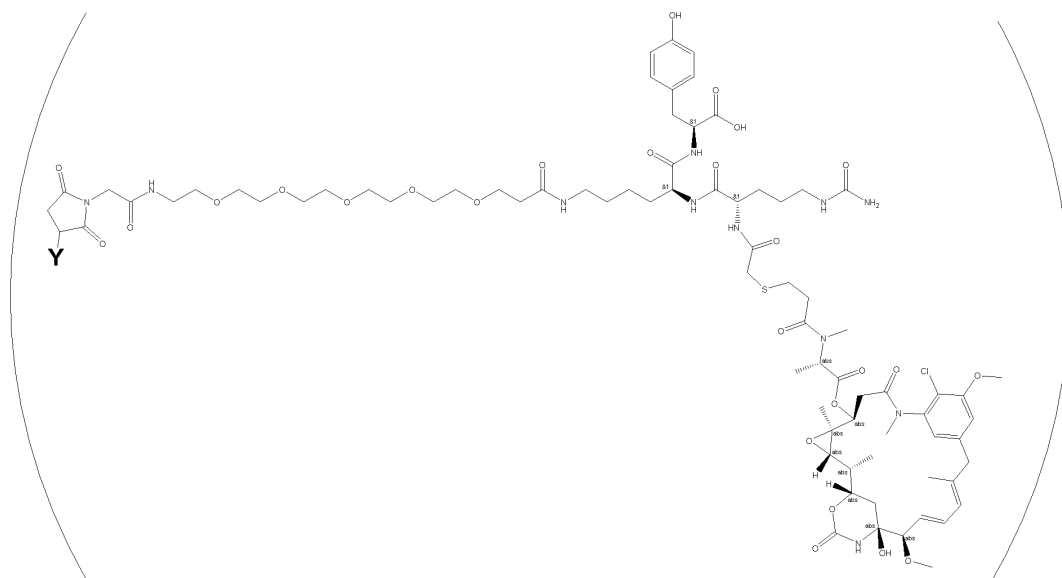




wherein **V** and **m** are as defined in any of the preceding claims and preferably **V** is naratuximab and **m** is 4 to 8,

20 with the proviso that **V** is to be understood as being outside the parentheses, such that **m** does not apply to **V** and with the proviso that the number of oxyethylene groups may also be selected from the ranges 12 to 30, preferably 14 to 25, more preferably 15 to 19,

25 or the compound of formula (I) is a compound wherein **V** is as specified in claim 1 and preferably derived from naratuximab and the moiety -LL-D is represented by the following formula:



8. The combination of claim 1, wherein the conjugate compound of formula (I) contains a linker LL that is selected from N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP); N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB) or N-succinimidyl 4-(2-pyridyldithio)-2-sulfobutanoate (sulfo-SPDB); N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC); N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfoSMCC); N-succinimidyl-4-(iodoacetyl)-aminobenzoate (SIAB); and N-succinimidyl-[(N-maleimidopropionamido)-tetraethyleneglycol] ester (NHS-PEG4-maleimide) and preferably SMCC or NHS-PEG4-maleimide.
9. The combination of claim 1 or 8, wherein the conjugate compound of formula (I) is naratuximab emtansine (Debio1562).
10. The combination of any one of claims 1 to 9, wherein the combination comprises a BCL2 inhibitor or pharmaceutically acceptable salt, solvate or polymorph thereof and the BCL2 inhibitor is selected from venetoclax (ABT-199), gossypol, sonrotoclax (BGB 11417), navitoclax (ABT-263), obatoclax (GX15-070), obatoclax mesylate, ABT-737, AZD4320, AZD0466, which is a conjugate of AZD4320 with PEG and a polylysine dendrimer, TW-37, antimycin A, apogossypolone (Apo-G2), HA14-1, and chelerythrine.
11. The combination of claim 10, wherein the BCL2 inhibitor is a BCL2-selective inhibitor, preferably venetoclax.

12. The combination of any one of claims 1 to 9, wherein the combination comprises an inhibitor of the PI3K pathway or pharmaceutically acceptable salt, solvate or polymorph thereof, and the inhibitor of the PI3K pathway is selected from a PI3K inhibitor that may for instance be selected from IPI-145, idelalisib (CAL-101), buparlisib (AN2025), roginolisib (MSC2360844), copanlisib (BAY 80-6946), IC-87114, PIK-93, pictilisib (GDC-0941), dactolisib (BEZ235), GSK1059615, BX-912, SF1126, pilarsilib (SAR245408), voxtalisib (SAR245409), BGT226, gedatolisib (PKI-587), NVPBE235, izorlisib (CH5132799), ZSTK474, sonolisib (PX-866), B591, TG100-115, RIDR-PI-103, alpelisib (BYL719), serabelisib (INK-117), GSK2636771, zandelisib (ME-401), AMG319, lisperlisib (YY-20394), parsaclisib (INCB050465), umbralisib (TGR-1202), PF-04691502, tenalisib (RP6530), taselisib (GDC-0032), AZD8186, AZD8835, duvelisib (IPI-145), leniolisib (CDZ173), eganelisib (IPI-549), apitolisib (GDC-0980), omipalisib (GSK2126458), samotolisib (LY3023414), bimiralisib (PQR309), paxalisib (GDC-0084), voxtalisib (XL765), SAR245409, VS-5584; 15

an Akt inhibitor that may for instance be selected from AT7867, MK-2206, perifosine (KRX-0401), triciribine, ipatasertib (RG7440), afuresertib (GSK2110183), uprosertib (GSK2141795), capivasertib (AZD5363), solenopsin, solenopsin analogues, HY-10249A, AT13148, KP372-1, GSK690693, erufosine, erucylphosphocholine, 20 ilmofofosine, edelfosine; or

an mTOR inhibitor that may for instance be selected from everolimus, temsirolimus, KU 0063794, AZD 8055, ridaforolimus (AP23573), umirolimus, zotarolimus, torin-1, sapanisertib (INK128), vistusertib (AZD2014), PP242, OSI-027, WYE354, WYR- 25 125132, INK128/MLN-0128, deforolimus, onatasertib (CC-223), as well as the dual PI3K/mTOR inhibitors such as dactolisib, pilarlisib, voxtalisib, apitolisib, gedatolisib, BGT226, GSK2126458, PF-04691502, VS-5584, SF-1126; and preferably idelalisib, IPI-145, IC-87114, PIK-93, GDC-0941, GSK1059615, KU0063794, AZD8055, MK-2206 or triciribine.

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13. The combination of any one of the preceding claims for use in the treatment of cancer.

14. The combination for use according to claim 13, wherein the cancer is DLBCL.

35 15. A conjugate compound of formula (I) or pharmaceutically acceptable salt, solvate or polymorph thereof, for use in a method of treating cancer, wherein the cancer is preferably DLBCL, and wherein the method comprises administering the conjugate compound of formula (I) or pharmaceutically acceptable salt, solvate or polymorph

thereof in combination with a co-agent selected from a BCL2 inhibitor or pharmaceutically acceptable salt, solvate or polymorph thereof and a PI3K pathway inhibitor or pharmaceutically acceptable salt, solvate or polymorph thereof,

- 5 wherein the conjugate compound of formula (I) is as specified in any one of claims 1 to 9 and wherein the BCL2 inhibitor or pharmaceutically acceptable salt, solvate or polymorph thereof and a PI3K pathway inhibitor or pharmaceutically acceptable salt, solvate or polymorph thereof is preferably as specified in any one of claims 10 to 12.

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